Proceedings of the 35th Annual Aquatic Toxicity Workshop: October 5 to 8, 2008, Saskatoon, Saskatchewan

Compes rendus du 35ième atelier annuel sur la toxicité aquatique: du 5 au 8 octobre 2008, Saskatoon, Saskatchewan

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Preface/Preface

The 35th Annual Aquatic Toxicity Workshop was held at the Delta Bessborough in Saskatoon, Saskatchewan, from October 5-8, 2008. The Workshop included three plenary presentations, 134 platform and 68 poster papers. Total attendance was 405.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 35ième atelier annuel sur la toxicité a eu lieu au Delta Bessborough, Saskatoon, Saskatchewan Octobre 5-8, 2008. L'atelier a donné lieu à 3 communications lors de séances plénières, 134 exposés d'invités d'honneur et 68 communications par affichage. 405 personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publies l'aide du ministre des Pêches et Océans.

Editors' comments/Remarques des editeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Graham, Ms. Monica Lyons, Ms. Fiona Price and Ms. Shandra Sedgewick for their assistance in the preparation of these proceedings.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longueur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs en chef voudraient remercier Dr. Jill Graham, Mme Monica Lyons, Mme Fiona Price et Mme Shandra Sedgewick dans la préparation de ces comptes rendus.

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Erratum **162**

The following paper was inadvertently omitted from the Proceedings of the 34th Annual Aquatic Toxicity Workshop , Halifax, NS: A Critical Review of the Environment Canada *Lemna minor* Biological Test Method EPS 1/RM/37 – March 1999. *D. Huebert* 162

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WINNERS OF DR. RICHARD PLAYLE AWARDS

Ecotoxicological assessment of juvenile northern pike inhabiting lakes downstream of a metal mine (PL)

J. Kelly[1]. [1]*University of Saskatchewan, Saskatoon, SK*

The objectives of this study were (1) to evaluate factors contributing to altered bioenergetics of juvenile northern pike *(Esox lucius)* inhabiting lakes receiving effluent from a uranium milling operation in northern Saskatchewan, and (2) to examine the potential effects of effluent exposure on biomarkers of oxidative stress and histopathology of target organs. Although glycogen and triglycerides stores were significantly greater in pike from exposure lakes compared to the reference lake, triglycerides stores of juvenile pike prey items showed no overall differences among lakes. Measures of parasitism, however, were negatively correlated with pike bioenergetics, thereby reflecting a possible energetic cost of parasitism on reference lake fish. The degree of infection by intestinal parasites and gill monogeneans was greatest in reference pike and intermediate in low-exposure pike, whereas high-exposure pike harboured no parasites. The potential for oxidative stress was assessed in pike liver and kidney using several biomarkers. Overall, the concentrations of total, reduced, and oxidized glutathione and the ratio of oxidized to reduced glutathione did not differ significantly among exposure and reference pike. The activity of glutathione peroxidase was greater in high exposure than reference liver, whereas lipid peroxidation was greater in reference than in exposure pike tissues. Trace element analyses of muscle showed that eight elements (arsenic, cobalt, copper, iron, molybdenum, selenium, thallium, uranium) were significantly elevated in exposure pike. These results provide only limited evidence of oxidative stress in exposure pike tissues and no evidence of histopathology, despite evidence that certain trace elements are bioaccumulating in tissue.

Characterization of OLGA PH J/92, a cell line derived from neural tissue of the freshwater crayfish, *Orconectes limosus*, and comparison with a goldfish brain cell line (PL)

M. Bufalino[1]. [1]*Wilfred Laurier University, Waterloo, ON*

Neural cell lines have been extremely useful for elucidating mechanisms of neural cell function, regeneration, and disease for mammalian species. In this study, we report on a newly established neural cell line from goldfish cerebellum (GFB3C) and compare growth characteristics in response to various parameters (temperature, osmolality, nutritional supplements, growth/differentiation factors and response to metals) with an aquatic invertebrate cell line (OLGA-PH-J/92) derived from the cerebral ganglia of crayfish. The OLGA cell line

grew best at 27 °C whereas GFB3C grew well at 22-30 °C. For both cell lines, best growth was observed in Leibovitz's L-15 medium supplemented with 10 % Fetal Bovine Serum, compared to other common mammalian or insect culture media. OLGA grew best at lower osmolalities than GFB3C cells, which was inconsistent with their freshwater invertebrate origins. Addition of glucose (L-15 contains galactose) or growth factors did not affect growth of either cell line. Formation of neurospheres, a characteristic of neural stem cells, was also investigated. OLGA cells did not form neurospheres, whereas GFB3C did quite readily. Immunostaining revealed that both cell lines consisted primarily of glial cells, as positive staining for glial fibrillary acidic protein, a glial cell marker, was found. The cells were tested for differentiation capabilities using retinoic acid or adhesion to poly-L-lysine as differentiation agents. Neuronal-like cellular processes could be induced when GFB3C neurospheres were plated onto poly-L-lysine coated plates. Finally, in terms of toxicity, both cell lines were highly tolerant to metal exposure, which correlates with their in vivo behaviour. Both could be useful for understanding mechanisms of neural growth and differentiation in vertebrate and invertebrate species, as well as in toxicity response and resistance in aquatic animals.

ENVIRONMENTAL EFFECTS MONITORING

Comparing the sub-lethal toxicity of thallium and effects of speciation and modifying factors in two aquatic species *(Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata)* (PL)

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Abstract

Thallium was recently proposed as a recommended effluent quality parameter under the metal mining effluent regulation (MMER) environmental effects monitoring program (EEM). Thallium has been shown to significantly increase in both water and aquatic biota after exposure to metal mine effluent, however, there is a lack of knowledge as to its fate and effect in the aquatic environment. The objective of this project was to assess (1) sub-lethal toxicity of the two main thallium species, thallium I and thallium III; and (2) potential modifying factors, such as potassium and calcium, for thallium I and III toxicity. IC25s were determined for both thallium species using the *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* sub-lethal toxicity tests. Thallium (III) was 25 times more toxic than thallium (I) for *C. dubia* and 260 times more toxic for *P. subcapitata*. Potassium had a protective effect on thallium (I) toxicity for both species; however, this effect was not as evident when tested with thallium (III). Results from this project will allow evaluation of thallium as a potential contaminant of concern for the mining industry, as well as provide important information regarding the risk assessment of thallium in metal mine effluent.

Introduction

Thallium is thought to be carcinogenic to humans and has been shown to be more acutely toxic than mercury, cadmium, lead, zinc and copper in mammals (Cheam, 2001). It is listed as a priority pollutant by the USEPA (Keith and Telliard, 1979). The aqueous release of thallium from metal mine effluents has been observed (Weber et al, 2008; Rickwood et al, 2008; Rickwood et al, 2006). Higher concentrations have been recorded in effluents from mines processing copper, lead and zinc bearing materials (Wallwork-Barber et al, 1985). Thallium was recently proposed as a recommended parameter under the metal mining effluent regulation (MMER) environmental effects monitoring program (EEM), based on reported discharges. However, studies assessing the toxicity of thallium to aquatic organisms are lacking, therefore assessing the relevance of thallium as a contaminant of concern for the mining industry is difficult. In addition, the majority of research conducted to date has been with thallium (I);

thallium exists primarily in two redox states (+1 and +3) in freshwater. As thallium (I) is more thermodynamically stable than Tl (III) it was thought to be the dominant form. However, recent studies assessing thallium speciation in the great lakes revealed that thallium (III) made up approximately 60 % of the total thallium measured (Lin and Nriagu, 1999). No sub-lethal data exists on thallium (III) toxicity to aquatic organisms.

Investigations into the influence of certain water quality criteria on the toxicity of thallium revealed that potassium has a protective effect on thallium (I) (Borgmann et al, 2004; Hassler et al, 2007). However, no data exists on whether a similar response is observed with thallium (III) or whether any other parameters have a modifying effect on thallium (III) toxicity. Due to this lack of knowledge, assessing the risk associated with thallium discharge in mine effluents is currently not possible.

Therefore, our objectives were two-fold;1) to conduct an exposure assessment to obtain inhibition concentrations (IC25) for two aquatic species; the algae, *Pseudokirchneriella subcapitata* and the invertebrate *Ceriodaphnia dubia* for both thallium (I) and (III) and 2) to conduct modifications on water quality to understand how certain water quality parameters can affect the toxicity of both thallium (I) and (III).

Methods

C. dubia were obtained from Aquatic Research Organisms (New Hampshire, USA) and *P. subcapitata* were obtained from the University of Toronto Culture Collection (Toronto, Ontario). Cultures were maintained at CANMET's metals and minerals science laboratories, Ottawa, ON. Reference toxicity tests were conducted with copper sulphate ($CuSO_4$) for *C. dubia* and zinc sulphate ($ZnSO_4$) for *P. subcapitata* within two weeks of each test to monitor responses of the organisms. *Thallium solutions and modifications*

Stock solutions (1 $g{\cdot}L^{-1}$) of thallium (I) nitrate and thallium (III) nitrate (Sigma Aldrich, analytical grade) were prepared with either 0.1 N HNO3 (Tl I) or 0.1 N HCL (Tl III) and milli-q water. Subsequent Tl (I) and Tl (III) dilution series were prepared with M4 stock solution (Elendt and Bias, 1990). Both *C. dubia* and *P. subcapitata* were exposed to seven concentrations of both Tl (I) (6.25 to 400 $\mu g{\cdot}L^{-1}$) and (III) (0.625 to 48$\mu g{\cdot}L^{-1}$) plus control M4 solution.

To assess the toxicity of thallium (I) and (III) under different water quality scenarios, M4 solution was modified by removing calcium ($CaCl_2$) and potassium (KCl) resulting in three solutions: 1) M4 solution 2) M4 solution reduced calcium 3) M4 solution reduced potassium. Total thallium concentrations of all dilution series were quantified by ICP-MS (Elan 6100 DRC).

Ceriodaphnia dubia

For detailed methodology please see Environment Canada protocol (EPS 1/RM/21, 2007). In brief, one organism was transferred into each of ten replicates per treatment and held in an incubation chamber with controlled temperature (25 °C ± 1 °C) and photoperiod (16 h L: 8 h D). Organisms were transferred daily into fresh solutions and any neonates were counted and then discarded. Dissolved oxygen, conductivity, pH and temperature were measured before and after daily transfer. Termination of the test occurred once organisms in the control treatments had produced three broods of neonates, usually within 6 days. Endpoints measured were number of neonates produced during exposure as well as survival of adults. Inhibition and lethal concentrations (IC25 and LC50 respectively) were calculated with measured concentrations using linear interpolation (CETIS version 1.025B database).

Pseudokirchneriella subcapitata

The growth inhibition test with the green algae *P. subcapitata* (formerly Selenastrum capricornutum) was based on the Environment Canada test method (EPS RM/25, 2007a), including the use of double deionized (milli-q) water spiked with nutrients as the exposure medium (M4 solution). In brief, 3-4 day old cultures were used with an initial cell density of 10,000 ± 1000 cells·ml^{-1} for each test well, with 4 test replicates for each of the 7 test concentrations. Multi-well plates were placed in an incubation chamber at 24± 2 °C with continuous lighting of 4000 ± 400 lux for 72 h. An electronic particle counter was used to measure cell concentrations at the end of the tests (Z2 Coulter® particle counter and size analyzer, Beckman Coulter Canada Ltd, Mississauga, Ontario). The 25 % growth inhibition concentrations (IC25) were calculated with measured concentrations using linear interpolation (CETIS version 1.025B database).

Results and discussion

Thallium (I)

IC25s determined for both *C. dubia* and *P. subcapitata* were160 μg·L^{-1} (95 % confidence intervals: 97 – 229 μg·L^{-1}) and 130 μg·L^{-1} (95 % confidence intervals: 116 – 147 μg·L^{-1}) respectively; these concentrations suggest similar species sensitivities. When we compare these results to previously determined IC25s for the same species, the levels are slightly higher. Pickard et al (2001) determined IC25s at 100 μg·L^{-1} (*C. dubia*) and 90 μg·L^{-1} (*P. subcapitata*) using thallium (I) in lab water.

When calcium was removed from the test solutions, IC25s increased slightly to188 μg·L^{-1} for *C. dubia* (95 % confidence intervals: 138 – 218 μg·L^{-1}) and 182 μg·L^{-1} (95 % confidence intervals: 133 – 280 μg·L^{-1}) for *P. subcapitata.* Despite this increase, confidence intervals were within the range of those recorded when calcium was present. This would indicate that the addition or removal of calcium had little effect on thallium (I) toxicity for these species. When potassium was removed from the test solutions IC25s decreased to 35 μg·L^{-1} for *C. dubia* (95 % confidence intervals: 27 – 40 μg·L^{-1}) and 4.59 μg·L^{-1} for *P. subcapitata* (95 % confidence intervals: 4 – 6 μg·L^{-1}); a chronic seven-day LC50 was also determined for *C. dubia* (71 μg·L^{-1}). These results indicate that potassium has a protective effect on thallium (I) toxicity for both the algal and invertebrate species (Figure 1). This response is similar to other studies conducted that looked at the influence of potassium concentrations on thallium (I) toxicity to a number of both terrestrial (Siegel and Siegel, 1975, 1976) and aquatic (Borgmann et al, 2004; Hassler et al, 2007) organisms. Both thallium and potassium share an almost identical atomic radii and ionic mobility, and thallium uptake via potassium pathways is thought to be the predominant route of exposure (Hassler et al, 2007). Therefore, in low concentrations of potassium, uptake of thallium would increase.

Thallium (III)

The toxicity of thallium (III) was substantially higher when compared with thallium (I). IC25s for *C. dubia* and *P. subcapitata* were 5.75 μg·L^{-1} (95 % confidence intervals: 5 – 15 μg·L^{-1}) and 0.519 μg·L^{-1} (95 % confidence intervals: 0.4 – 0.7 μg·L^{-1}) respectively. While IC25s for thallium (I) were similar for both species, inhibition concentrations were approximately 30-fold lower for *C. dubia* and approx 340-fold lower for *P. subcapitata*, indicating that the algal species had increased sensitivity to thallium (III) toxicity compared to the invertebrate *C. dubia*.

When calcium was removed a similar IC25 was calculated for *P. subcapitata* at 0.331 μg·L^{-1} (95 % confidence intervals: 0.3 – 0.4 μg·L^{-1}) suggesting that calcium had no effect on the toxicity of thallium (III) (Figure 2). Interestingly, an IC25 could not be calculated for *C. dubia* as no inhibition in reproduction occurred. This is an interesting finding and would suggest that the presence of calcium actually increased the toxicity of thallium (III) to this species. It is unclear what mechanisms could be behind such a response and further investigation is required.

When potassium was removed from the test solutions the IC25 for *C. dubia* decreased to 0.511 μg·L^{-1} (95 % confidence intervals: 0.3 – 12 μg·L^{-1}) and a chronic LC50 was determined (41.65 μg·L^{-1}) (Figure 2), demonstrating a similar response to thallium (I). However, the IC25 for *P. subcapitata* increased to 46.40 μg·L^{-1} (95 % confidence intervals: 42 – >48 μg·L^{-1}), suggesting that the presence of potassium increases the toxicity of thallium (III) to this species. Again, it is unclear as to the mechanisms behind this response but further investigation is underway.

Very little data exists on the toxicity of thallium (III), making comparisons to previous studies difficult. An acute exposure of thallium (III) was conducted on *Daphnia magna* where an LC50 was determined at 24 μg·L^{-1} (Lan and Lin, 2005). An LC50 was not observed in our study even though concentrations exceeded 24 μg·L^{-1}. Again, this could primarily be due to species differences and/or water quality; when potassium was removed from our control water an LC50 of 41.64 μg·L^{-1} was observed.

In conclusion, thallium (III) is substantially more toxic than thallium (I) for both species. Potassium had a protective effect on toxicity of Tl (I) for both *P. subcapitata* and *C. dubia*. However, the same cannot be said for thallium (III), where the absence of potassium decreased toxicity to *P. subcapitata.* Therefore, the mechanisms by which potassium alters the toxicity of thallium (I) may not apply to thallium (III) in some species. Further investigation will be conducted to try to identify parameters that could alter the toxicity of thallium (III); this would not only aid in conducting an appropriate risk assessment but also help to understand the mechanisms behind its toxicity.

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Figures

Figure 1. Comparison of inhibition concentrations (IC25; μg·L^{-1}) for thallium (I) for both *P. subcapitata* and *C. dubia* with no modifications (none), removal of potassium (K+) and removal of calcium (Ca2+). Error bars are 95 % confidence intervals.

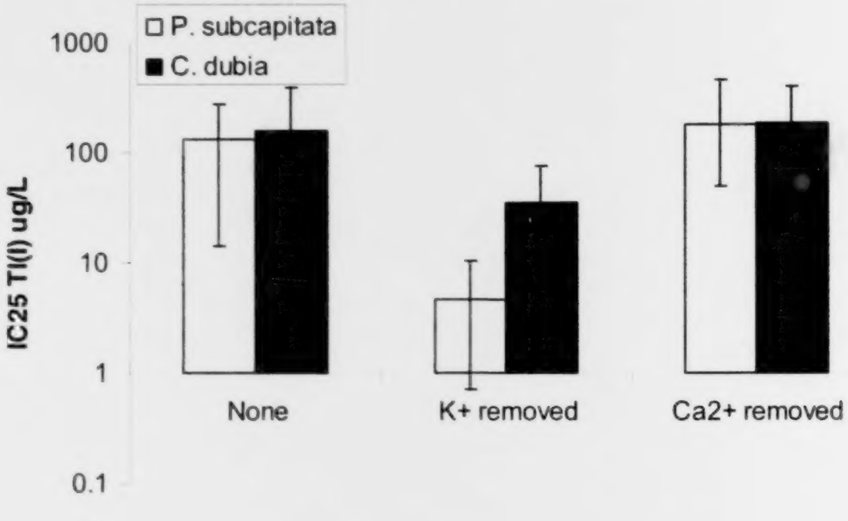
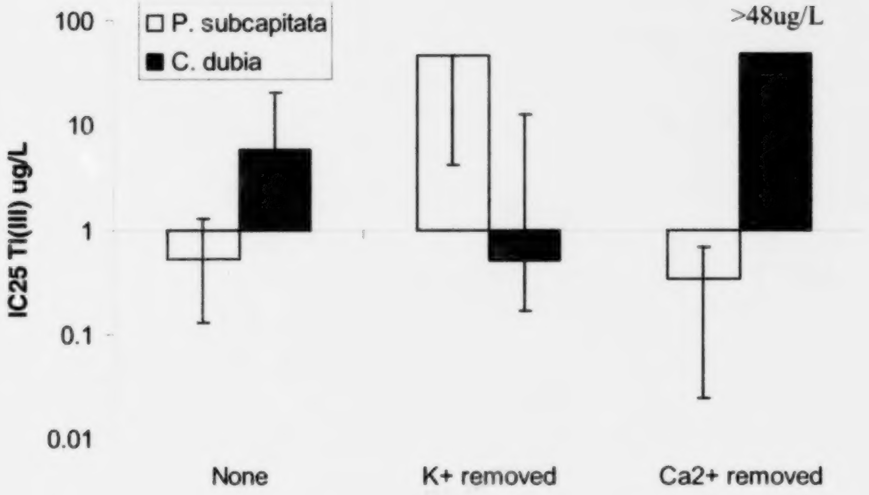


Figure 2. Comparison of inhibition concentrations (IC25; μg·L^{-1}) for thallium (III) for both *P. subcapitata* and *C. dubia* with no modifications (none), removal of potassium (K$^+$) and removal of calcium (Ca^{2+}). Error bars are 95 % confidence intervals.



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Long-term patterns of effects of pulp and paper mill effluents on fish and invertebrates: potential for improvements on a national scale (PL)

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The National Environmental Effects Monitoring (EEM) Program has recently completed the fourth cycle of pulp and paper mill effluent monitoring. Meta-analyses were used to evaluate national response patterns over the last decade for fish and benthic invertebrates exposed to mill effluent. In general, response patterns for fish and invertebrates in Cycle 4 were quite similar to those observed in earlier cycles, and were typical of nutrient enrichment conditions co-occurring with reduced fish gonadal growth. A superficial look at the fish results might suggest significant lessening of effects in Cycle 4, relative to earlier cycles. This, however, was mostly a result of the bias introduced into the analyses due to many of the large-effect mills going to Investigation of Cause (IOC) studies in Cycle 4, and therefore not submitting standard survey data that could be included in the meta-analyses. After correcting for this bias, the apparent reduction in effects mostly disappeared. The notable exception was relative gonad size, which showed a significant lessening of the national average effect, even after correcting for the bias. An interesting side effect of looking at national response patterns for fish in Cycle 4, without correcting for the IOC bias, was to show what the pulp and paper industry as a whole could achieve if mills having large effects are successful in fixing their effects. In contrast, a larger number of mills would have to lessen their effects on invertebrates before a similarly large industry-wide improvement would be achieved for benthic invertebrate communities.

Alternative to fish surveys studies in Environmental Effects Monitoring (EEM) pulp and paper cycles 2-4 (PL)

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Alternative to fish survey studies have been historically used in such situations as hazardous conditions, unsuitable habitat for sampling, presence of confounding factors and repetitive failure to collect appropriate samples. Renewed interest in the alternative to fish survey studies comes from the Smart Regulations Process for pulp and paper that recommended flexibility in moving forward to Investigation of Cause (IOC) studies. Some concerns were expressed that certain alternative tests have not been very satisfactory in terms of relevant findings, costs and/or the level of effort required. We present the findings of a review of alternative methods that was conducted to assess the basis of these concerns and recommend next steps in development and application of alternative tests for the EEM Pulp and Paper program. Work continues to increase the array of recommended methods, correlate the responses (standard surveys vs. alternative tests) where possible, and to use tests that are focused on responses judged to be of high ecological and societal importance and that are able to measure required endpoints by non-standard means.

Reducing variance and increasing power of a reproductive bioassay by understanding the spawning behaviour of mummichog *(Fundulus heteroclitus)* (PL)

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The mummichog (*Fundulus heteroclitus*), an abundant, small-bodied estuarine fish, has been widely used in the Environmental Effects Monitoring program in periodic monitoring and investigation of cause (IOC) studies to determine the impact of pulp and paper mill effluents on fish reproduction. Recent studies using an adult mummichog reproduction bioassay demonstrated relatively high variance and low power to detect differences in cumulative egg production in response to exposure to thermomechanical pulp mill effluent. This prompted further investigations on mummichog reproductive biology in the lab, in order to increase the power of the reproductive bioassay. A series of studies in the southern (U.S.) subspecies have shown that mummichog (*F. h. heteroclitus*) egg production is lunar, with peak reproductive output occurring at full and new moons. Little is known about reproductive biology of the northern mummichog variety found in Canadian estuaries (*F. h. macrolepidotus*), but recent studies indicate that temperature may be a dominant cue for reproduction in the Bay of Fundy and not the lunar cycle. We collected northern mummichog close to the upper part of its geographic range in the Northumberland Strait, New Brunswick (46°20' N, 64°40' W). Under laboratory conditions, eggs were collected every two days for a period of two months. The population spawned daily and with considerable variance. Peak productivity in egg production was during the week leading up to the new moon, while lowest production occurred around the full moon. This indicates a weak lunar pattern in reproductive activity. Variance in cumulative egg production decreased three-fold in the initial 14 days of egg production and remained stable afterwards. Given this variability and weak lunar pattern, we are currently evaluating options for further optimizing power in the reproductive bioassay.

Analysis of environmental effects monitoring data: dealing with heterogeneous regression slopes in analysis of covariance (PL)

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Analysis of covariance (ANCOVA) is a powerful statistical method which incorporates one or more covariates into the analysis to reduce error associated with measurement. ANCOVA (modeling response as a function of fish size) is frequently used to analyze environmental effects monitoring (EEM) fish survey data. In approximately 11 % of fish survey data sets taken from cycles 1 to 3 of Environment Canada's EEM database for pulp and paper mills, the standard assumption of parallel regression slopes is not met. These data sets are currently classified as indicating a mill effect, but are excluded from subsequent analyses aimed at quantifying the effect. We present two different methods for dealing with data sets that exhibit heterogeneous slopes. The first method identifies data sets where heterogeneous slopes are forced by a few high-influence observations. The second approach identifies data sets where a model with heterogeneous slopes is statistically, but not practically, significant: with a large coefficient of determination for the parallel slope model. When these methods are applied to the EEM pulp and paper data sets, about 55 % of cases which were previously excluded can be included in analyses. We also suggest a method of describing mill effects when regression slopes are heterogeneous, enabling comparison with a critical effect size. These methods enable more data sets to be incorporated into meta-analyses and will lead to more equitable mill monitoring protocols in the future.

From investigation of cause to investigation of solutions: nutrient-related EEM studies of the Wapiti River (PL)

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Previous EEM studies have documented nutrient-related enrichment of receiving environments downstream of several Canadian pulp mills. In some cases, the study of these enrichment effects has been confounded by municipal sewage discharges to the same river reach. For the Wapiti River near Grande Prairie, Alberta, previous EEM Investigation of Cause studies - which used field, mesocosm, and modeling approaches - have indicated that downstream algal biomass increases mainly because of nitrogen discharged in municipal sewage and phosphorus discharged in pulp mill effluent. We present a proposed Investigation-of-Solutions study for Grande Prairie EEM Cycle Five, which focuses on in-mill strategies to reduce phosphorus discharges to the Wapiti River. These include optimization of the effluent treatment system, as well as reducing phosphorus needed for treatment through potential reductions in influent flow and BOD loading to the treatment system. The potential effect of predicted phosphorus reductions on in-river algal biomass will be assessed using numerical models of algal response to nutrients developed in EEM Cycle Four.

National water quality and effluent characterization trends of Canadian metal mines and benthic community response (PL)

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The National Environmental Effects Monitoring (EEM) Office has recently compiled the environmental data collected under the Metal Mining Effluent Regulations dating from to 2003 to 2006. National effluent concentration averages have been calculated describing the differences and similarities between precious metals, base metals, iron, and uranium mining operations on an annual and time-span basis. Also, the water quality of receiving aquatic environments was compared to the water quality of reference sites. Changes in water quality were correlated to changes in benthos, illustrating relationships of statistical significance. The importance of choosing an appropriate time span for water quality analysis was examined. Together, the statistical summary of effluent discharge and the correlation between water quality and benthos can be useful tools for characterizing the effluent-associated impacts of metal mines, identifying areas of environmental improvement, and focusing the direction of future research.

Effects of mining effluent on ninespine stickleback liver and gonads (PL)

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Miramar Northern Mining Ltd.'s Con Mine Ltd. has undertaken two phases of Environmental Effects Monitoring (EEM) of fish and benthic invertebrates in the Yellowknife area. Treated effluent is discharged from the water treatment plant and ultimately enters a small, narrow bay called Jackfish Bay on Great Slave Lake. The total length of the area exposed to treated effluent is approximately 7 km. Water quality parameters that are of potential environmental concern are chloride, cyanide, ammonia, and nitrate, and several metals including arsenic, copper, lead, nickel, zinc, and strontium. In 2004 and in 2007, ninespine stickleback were studied in areas exposed to effluent and areas away from effluent. Results showed that liver and gonads were different between the exposure and reference areas; gonads and livers were substantially larger in the areas exposed to effluent. In general, though, there is no direct evidence that these effects have impacted the ninespine stickleback's ability to survive or reproduce in the exposure area. Stickleback have proven to be a good species for ecotoxicological studies and more baseline data collection in the north is required.

An assessment of environmental effects monitoring program endpoints in a baseline environment: are effects found prior to mine effluent release? (PL)

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Cameco Corporation and its joint venture partners (AREVA and JCU) have recently completed a pre-feasibility study for the proposed development of a uranium mine at the Millennium deposit located in northern Saskatchewan. As part of the studies conducted to characterize the existing environment at the Millennium site, an Environmental Effects Monitoring (EEM)-based program was performed to establish a baseline for future EEM studies. Benthic invertebrate community and fish population surveys were conducted at the proposed future exposure site in Moon Lake and at a reference site in Slush Lake. Site characterization information demonstrated that Slush and Moon lakes have similar habitats, water and sediment chemistry, and fish communities. The results of the EEM-based survey found effects, as defined by the EEM program, on fish and the benthic invertebrate community in Moon Lake. All EEM effect endpoints for the benthic invertebrate community survey were significantly different between the study areas. Results from the northern pike non-lethal fish population survey showed that young-of-the-year fish were significantly larger in Slush Lake than in Moon Lake. The lethal yellow perch study found that male fish from Moon Lake had significantly larger size-at-age, while female fish from Slush Lake had significantly larger relative gonad and liver sizes. These results show that EEM-defined effects can occur naturally in the environment between two similar un-impacted waterbodies. This stresses the importance of using multiple reference sites and an appropriate effect size for determining mine-related impacts, since statistical differences do not equate to ecological effects.

Effects on the benthic invertebrate community downstream of Giant Mine (PL)

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Giant Mine is an abandoned gold mine located approximately five kilometres north of the City of Yellowknife, Northwest Territories. In 1999, the former owner of the Mine was assigned into receivership and the Government of Canada began to manage care and maintenance of the Mine. Continued seasonal operation of the effluent treatment plant is necessary to remove arsenic from groundwater that infiltrates the Mine and becomes contaminated. Water level in the Mine is controlled to prevent arsenic trioxide storage chambers from flooding and potentially releasing arsenic into the environment. After treatment, effluent is discharged to Baker Creek and Yellowknife Bay (Great Slave Lake). This practice will continue until a long-term management method for stored arsenic trioxide is implemented and Mine water quality meets discharge criteria. Prior to the implementation of effluent treatment in the 1980s, benthic invertebrates were virtually absent from Baker Creek due to high contaminant concentrations. Invertebrate re-colonization within Baker Creek was documented in 2002. The effects of present day effluent discharge on the invertebrate community in Baker Creek and Yellowknife Bay were assessed in 2004 and 2006 using artificial substrate samplers (Hester-Dendy multi-plate samplers). Analysis of data collected in 2004 and 2006 highlights the low level of effects observed on artificial substrates deployed in the water column, in comparison to the severe effects observed in bottom sediments by previous studies. These results suggest that historical sediment contamination likely poses a greater risk to aquatic life in Yellowknife Bay than the periodic discharge of treated mine effluent.

The influence of subsampling on the ability to detect effects in surveys of benthic macroinvertebrates (PL)

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The federal EEM program generally requires (some exceptions) that surveys of benthic macroinvertebrates include nested subsamples within stations. Proponents are required to collect enough subsamples to ensure that key indices of composition (i.e., abundance, richness) are estimated to within 20 % of their true mean values (at the station), i.e., a within-station precision of 0.2 or less. It is true that subsamples will increase the ability of a study to document an effect (statistically), but the influence of subsampling on the statistical power of a BIC study has not yet been demonstrated. We worked through a simulation exercise that varied the true within-station precision as well as sample size, and determined the probability of detecting an effect size of 2 SD's (i.e., the difference between reference and exposure locations was equal to two times the within location between station - standard deviation). The likelihood of declaring there to be a difference (in the simulation experiment) varied little whether one or multiple subsamples were collected within stations. Though subsamples may not be necessary in order to detect effects, they are still of value when the numbers of organisms per sample is very low.

The reference-degraded continuum: a new multivariate approach to assess environmental condition (PL)

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The current paradigm of environmental assessment entails comparison of a test site to the reference condition, whose environmental limits are determined empirically by intensive sampling of reference sites. Typically, the reference condition is characterized by the physicochemical characteristics of the best available sites and their associated biota. However, the relative status of test sites that are designated nonreference is undefined because of the binary nature of the classification. We operationally defined the degraded condition as comprised of sites whose physicochemical characteristics are deemed unacceptable by consensus. Consequently, any test site can be ordinated along a reference-degraded continuum, and its relative quality summarized by its position along the continuum. Two alternative stress-response patterns may be postulated when environmental quality of sites is plotted against a reference-degraded continuum scale. The degree of stress may determine response of the dependent variable (i.e., stress is a dominant and dose-like factor, best analysed by linear or logistic regression) or stress may limit the response of the dependent variable (i.e., stress is one bounding factor among many, best analyzed by quantile regression, which defines upper and lower bounds). Using the reference-degraded continuum conceptual model we have found that biological condition is frequently a nonlinear function of environmental stress that can be better modeled by piecewise quantile regression than by linear or nonlinear models. Threshold responses may be much more common manifestations of environmental degradation than gradual changes in biological condition. We illustrate these patterns with examples of fish assemblages at Great Lakes coastal margins, zoobenthic assemblages in the Lake Huron-Lake Erie Corridor, and terrestrial species richness in wetlands of varying areas.

What level of concern is warranted with respect to release of production waters on the Grand Banks of Newfoundland? (PL)

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The offshore oil and gas industry which is developing off the east coast of Canada will likely continue to garner attention in relation to its potential for effects on fisheries and the environment. The Terra Nova, Hibernia, White Rose, and Hebron fields are situated on important fishing grounds in a relatively small area on the northeast edge of the Grand Banks of Newfoundland. What will be the scale and nature of any impacts at individual development sites? The majority of information available from field and laboratory studies suggests that offshore impacts will likely be minimal with little potential for any impacts beyond individual rig sites. It is also reasonable to suggest that fishery closure zones around rigs could on occasion act as nursery areas or "marine protected areas". However general statements should always be approached with caution and it is important to point out that some uncertainties still exist about the effects of oil development, including the potential for chronic effects of produced water on fish and other biota. Recent laboratory and field studies carried out under the PERD program to 2008 will be reviewed. Regarding EEM programs by industry, the present programs on the Grand Banks are geared towards providing early warning of any impacts on fish health, fish quality, sediment toxicity, and primary productivity. Such programs will continue to be important for assurance or otherwise keeping a check on things we think we know. EEM programs also provide the means for possibly uncovering new insights which is not possible through laboratory approaches.

ENVIRONMENTAL EFFECTS MONITORING

Assessment of toxicity of upper Danube River sediments using a combination of chemical fractionation, the *Danio rerio* embryo assay and the Ames-fluctuation test (PO)

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The world's river systems provide fresh water to people and support thousands of species. However, many of the great rivers have been polluted throughout the past decades. Possible sources of such pollution include effluents from domestic sewage pants (e.g., urine and feces, detergents, pharmaceuticals), industry (e.g., PCBs, dioxins, and metals), agricultural runoff (e.g., pesticides and fertilizers), and storm water runoff from urban areas (e.g., salts, oil, and antifreeze). Severely contaminated sediments from many rivers and lakes have been shown to be acutely and chronically toxic to fish and benthic invertebrate species. For example, sediment samples from the Upper Danube River that were analyzed in six separate assays were found to have considerable geno-toxic, cytotoxic, mutagenic, embryo-toxic and estrogenic effects. It has been hypothesized that decline in fish stocks in the Upper Danube River since the early 1990s may be associated with this pollution. Here we report on the results of a study conducted to determine the toxicity of extracts from sediments of the Danube River by means of the *Danio rerio* embryo assay, and by assessing lethal and sublethal endpoints. In addition, mutagenicity was assessed using the Ames-fluctuation assay. For the sediment samples that revealed toxicity, fractionation of each sample was performed by separating compounds according to their polarity, planarity, and the size of the aromatic ring system. 18 fractions for each sediment sample were tested separately in the Ames-fluctuation assay and *Danio rerio* embryo assay to assess which group of chemicals within the sediment sample caused the original toxicity.

Identifying the toxic constituent: size really does matter (PO)

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A forensic investigation was conducted on an effluent pond water from a chemical plant in response to a failed monthly bioassay. The forensic investigation was designed to isolate, characterize, and identify the pond water constituents toxic to trout and *Daphnia*. Aliquots of the toxic sample were pH adjusted (acidic, ambient, and basic) then separately aerated, filtered, and subjected to solid phase extraction (C18 column). There were eight treatments at each of three pH's for a total of 24 fractions for testing. The fractions were screened for toxicity with the luminescent bacterium *(Vibrio fischeri)*, *Daphnia*, and fathead minnow (surrogate for trout). The partitioning of effects amongst the fractions was similar for all three species. A mass balance on toxicity gave recoveries of 22 % and 29 % at ambient pH and pH 9, respectively. Attempts were made to remove the balance of toxicity from the C18 columns, but no further toxicity was recovered. The plant provided information on an inadvertent release of a cationic coagulant into the pond. The coagulant was toxic to trout. Size exclusion testing was conducted on the toxic effluent sample. The toxic constituents could not pass through dialysis tubing with a pore size of 10,000 Daltons. These results confirmed that the toxic constituent present in the pond water was a large molecular weight compound like the coagulant (>80,000 Daltons). The compound would be removed by the C18 column (low recoveries) and would not be detected by conventional GC/MS (too big). The compound would not have been detected using conventional methods for identifying toxic constituents.

Bioindicator and fish health studies around the Terra Nova oil development site on the Grand Banks (PO)

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Bioindicators or health effect indicators have potential to identify adverse health conditions in fish, in advance of effects on populations. Thus they can be a valuable reconnaissance tool for addressing concerns of a real or perceptual nature on the part of fishing-industry and public interests about the scope of any potential impacts of contaminants on fish stocks. American plaice *(Hippoglossoides platessoides)* was an important commercial flatfish on the Grand Banks and was initially chosen by the oil industry in consultation with the Department of Fisheries and Oceans as an indicator species for Environmental Effects Monitoring programs in the area. We report here on fish health studies carried out at the Terra Nova development site before and after release of produced waters, which began in 2003. These studies constitute one component of the overall Terra Nova Environmental Effects Monitoring program. Fish were collected in the near vicinity of the development area (Study Area), as well as in a Reference Area located approximately 20 km southeast of the development. The health effect indicators studied included fish condition, visible skin and organ lesions, levels of mixed-function-oxygenase (MFO) enzymes, haematology (differential cell counts) and a variety of histopathological indices in liver (e.g. nuclear pleomorphism, megalocytic hepatosis, foci of cellular alteration, macrophage aggregation, neoplasms) and gill (e.g. hyperplasia, oedema, fusion and telangiectasis). These indicators have been extensively used in laboratory and field investigations with various fish species. Although a slight elevation of MFO enzyme activity was observed in fish from the Study Area in 2002, before release of produced water, and in 2006, other indices were similar between the Study and Reference Area, and overall results do not indicate any project effects.

ENDOCRINE MODULATING SUBSTANCES

Estrogenic and anti-estrogenic activity of wood extractives present in pulp and paper mill effluents evaluated in rainbow trout (PL)

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Wood extractives are constituents of wood present in pulp and paper mill effluents, which may cause reproductive disturbances in fish. A chronic-exposure toxicity experiment using immature rainbow trout (Oncorhynchus mykiss) was conducted in order to assess (anti)estrogenic potencies of the wood extractives dehydroabietic acid (DHAA) and β-sitosterol (BS), a model estrogen 17β-estradiol (E2) and two Chilean pulp and paper mill specific extracts (Solid Phase Extraction, SPE) obtained from primary and secondary treated effluents. The protocol involved the use of multiple intra-peritoneal injections (4 injections every 7 days for a total exposure period of 28 days), corrected for individual fish weight and based on previously determined dose information. Estrogenicity was measured as plasma vitellogenin (VTG) level. The phytosterol BS, E2 and both pulp mill effluent extracts had statistically significant increases in VTG levels after 4, 7, 14, 21 and 28 of exposure. While a weak statistically non-significant VTG level increment was observed in DHAA injections, simultaneous injection of DHAA and E2 reduced the VTG levels found in E2 injected fish, indicating an anti-estrogenic effect. Primary treated effluent extract injected fish showed a similar but delayed induction in VTG levels compared to fish injected with secondary treatment effluent extract, indicating a differential endocrine disruption effect due to the effluent treatment. Additionally, significant changes were observed in the gonad somatic index, these results are being confirmed by histology and inmmunohistochemical analysis of VTG of gonad.

Reproductive effects of waste water treatment plant effluent in fathead minnow, *Pimephales promelas* (PL)

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Pharmaceuticals have been detected in the rivers of Southern Alberta, a semi-arid region with intensive agricultural operations and a growing human population. The objective of this study was to characterize the effects of effluents from wastewater treatment plants (WWTP) on the reproductive status of fish sampled in the field and fish exposed to WWTP effluent in the laboratory. The fathead minnow, a sentinel species, was sampled upstream and downstream of WWTPs in the Oldman and South Saskatchewan rivers in 2006 and 2007. In the laboratory, adult fish were exposed to 10, 25, 50 and 100 % of WWTP effluent, or 10 ng·L^{-1} EE2 (ethinyl estradiol) and ethanol as positive controls for 21 days. Biochemical and morphological endpoints were measured to characterize the reproductive status; histology of gonads was used to determine sex, gonadal maturity and intersex. Liver vitellogenin (Vtg) mRNA, a biomarker of exposure to estrogenic chemicals, was analyzed using quantitative RT-PCR. Vtg mRNA was elevated in fish from some downstream river sites. An induction of Vtg was also detected in fish exposed to WWTP effluent in the laboratory. Preliminary results indicate fathead minnows in Southern Alberta are impacted by anthropogenic estrogenic chemicals. (Funded by CWN, the City of Lethbridge and NSERC).

Forensic ecotoxicology: in search for endocrine disrupting compounds in municipal wastewater (PL)

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We present a bioassay directed forensic approach to isolate and identify endocrine disrupting compounds present in treated municipal effluent. Biological screening for endocrine disrupting compounds was performed using yeast cells (yeast estrogen screen, YES, and yeast androgen screen, YAS) as well as the Fathead minnow. Endpoints monitored following exposure to Fathead minnow included: total plasma protein, vitellogenin induction (in males), gonad somatic index and secondary sexual characteristics. Municipal effluent samples were collected from the Gold Bar Wastewater treatment plant on two occasions and were found to illicit an estrogenic response using the YES and Fathead minnow assays. In order to identify the causative agents for such responses a forensic approach was applied. This involved passage of municipal effluent through solid phase extraction cartridges. Following extraction of 2 – 100 L of effluent, the cartridges were stripped with water alcohol mixtures. These mixtures were subjected to bioassay testing for endocrine responses. An estrogenic response was observed following extraction of treated wastewater using solid phase extraction and elution of the t-C18 extraction cartridge with 50 % methanol in water. This fraction was subjected to chemical analyses using gas chromatography/mass spectrometry following alkylation with diazomethane. A unique series of homologues typified by m/z 207 and 251 were observed. Interpretation of mass spectral data using first principles identified these compounds to be carboxyalkylphenol monoethoxycarboxylates (CAP1ECs) and carboxyalkylphenol diethoxycarboxylates (CAP2Ecs).

The major metabolites found in the 50 % MeOH extract were CA8P1EC, CA6P2EC and CA8P2EC. These are dicarboxylates formed by carboxylation of the alkyl side chain and the terminal ethoxy group in the nonylphenol ethoxylates (NP1EO and NP2EO). The dicarboxylates have been reported in treatment plant effluents and rivers but their endocrine disrupting properties have not been assessed. Our finding suggests that these compounds may be responsible for observed endocrine responses in treated municipal effluent.

Physiological and reproductive changes in fathead minnows (*Pimephales promelas*) resulting from short-term exposure to untreated and treated pulp and paper mill effluent (PL)

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As part of their manufacturing process, pulp and paper mills release effluents into waterways that may affect the fecundity, morphology, and physiology of invertebrates and vertebrates in the receiving ecosystem. Though these effluents are first treated, they may still negatively impact the aquatic environment. Therefore, there is a need to understand and quantify the impacts of these effluents on target aquatic species, as well as assess the effectiveness of effluent treatment on removing impacts of these effluents on fish reproduction. We used a short-term fathead minnow reproduction test (including a 15-day pre-exposure period and a 6-day exposure period) in order to determine reproductive and physiological changes resulting from exposure to three effluents from a kraft and news mill in Northern Ontario: 10 % (v/v) untreated kraft mill effluent (UK), 25 % (v/v) secondary (biologically) treated kraft mill effluent (SK), and 100 % (v/v) combined mill outfall (CMO). Egg production, behaviour, colouration, and individual endpoints were compared between treatment groups. Significant decreases in egg production occurred in UK-treated fish, and UK and SK exposure each caused masculinization in a female fathead minnow. Thus, while clarification and secondary effluent treatment appeared to improve the short-term reproductive impacts on fathead minnows observed in kraft mill effluent, these processes did not entirely remove toxicants causing masculinization. In contrast, CMO did not cause any reproductive or physiological changes in fish. This work is being extended with gene expression studies to determine the biochemical pathways impacted by exposure to pulp and paper mill effluent.

Real-time PCR gene expression analysis in liver of fathead minnows (*Pimephales promelas*) exposed to pulp and paper mill effluents suggests the presence of steroid mimics after activated sludge treatment (PL)

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Though exposure to pulp and paper mill effluents may induce physiological and morphological changes in fish, the molecular events involved have not been explored. We exposed sexually mature fathead minnows (FHMs) to three effluents from a kraft and news pulp and paper mill in northwestern Ontario for 6 days: 10 % (v/v) kraft effluent prior to its biological treatment (UTK), 25 % (v/v) biologically treated kraft effluent (TK) and 100 % (v/v) combined mill outfall (CMO). The expression of five genes responsive to endocrine disrupting compounds (EDCs) and oxidative stress was examined in liver versus river water controls using real-time PCR. In female FHMs, the mRNA levels of androgen- and estrogen-receptor β (AR and ERβ, respectively) in UTK- and TK-exposed fish and of CYP1A in UTK-exposed fish increased, though these changes were not statistically significant; exposure to CMO did not induce significant expression changes in any genes investigated. By contrast, in male FHMs there was significant upregulation of AR, ERβ and CYP1A in UTK- and TK-exposed fish, but no changes in ERα or vitellogenin expression. Finally, exposure to 100 % CMO significantly increased the mRNA levels of ERα, vitellogenin and CYP1A. Together, these results suggest that kraft effluent before and after biological treatment contained compounds able to induce androgenic effects in FHMs. Moreover, CYP1A upregulation suggests that potential toxicants were present in all three effluents, and that combination of kraft and newsmill effluents eliminated the androgenic compounds by dilution while inducing significant gene expression changes that may be due to estrogenic compounds produced by the newsmill.

Application of a medaka HPG axis real time PCR array method to chemical screening (PL)

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A real time polymerase chain reaction (RT-PCR) array was developed for studying chemical-induced effects on gene expression of selected endocrine pathways along the hypothalamic-pituitary-gonadal (HPG) axis of the small fish model, the Japanese medaka (*Oryzias latipes*). The Japanese medaka HPG PCR array combines the quantitative performance of SYBR® Green-based real-time PCR with the multiple gene profiling capabilities of a microarray to examine expression profiles of 36 genes associated with endocrine pathways in brain, liver and gonad. A pathway-based approach was implemented to analyze and visualize time -dependent or concentration –dependent mRNA expression in the HPG axis of Japanese medaka. The performance of the Japanese medaka HPG PCR array was evaluated by examining effects of five model compounds, the synthetic estrogen, 17α-ethinylestradiol (EE2), the anabolic androgen, 17β-trenbolone (TRB), the aromatase inhibitor, fadrozole (FAD), the imidozole-type fungicides, prochloraz (PCZ) and ketoconazole (KTC) on in four-month-old the Japanese medaka. The organ- gender- and concentration –specific gene expression profiles derived by the Japanese medaka HPG axis RT-PCR array provides a powerful tool to delineate chemical-induced modes of action. In addition, quantitative investigation revealed that the five egg precursors and ER-α in livers of females was log-log related to the ecologically relevant endpoint, fecundity.

Evidence for compensatory responses at the molecular, biochemical, and tissue level in fathead minnows exposed to steroidogenesis inhibitors. (PL)

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In order to survive, organisms require a capacity to adapt to a wide variety of biotic and abiotic stressors, including chemicals of both natural and synthetic origin. Recent studies in our laboratory have provided evidence of compensatory responses to endocrine active chemicals that act by inhibiting one or more enzymes involved in steroid biosynthesis. Specifically, we conducted a series of experiments in which fathead minnows were exposed to either the aromatase inhibitor, fadrozole, or the fungicide, ketoconazole, for varying durations (24 h to 21 d). Endpoints including gonadal mRNA transcript abundance, ex vivo steroid production, plasma steroid and vitellogenin concentrations, gonad histology, and reproductive success (fecundity) were examined. After just 24 h of exposure, fadrozole caused concentration-dependent decreases in both ex vivo estradiol (E2) production and plasma E2 concentrations in exposed females. However, by 8 d E2 concentrations had recovered to control levels. This recovery coincided with increased transcription of mRNAs coding for aromatase, P450 cholesterol side chain cleavage, and steroidogenic acute regulatory protein. Similarly, while exposure to ketoconazole reduced the rate of steroid production by fathead minnow testis tissue, it also elicited an overall increase in testis size relative to body mass and proliferation of the steroid producing interstitial cells, which appeared to offset the direct effect of the chemical.

These studies provide insights into compensatory responses at multiple levels of biological organization that have relevance to both exposure assessment and predictive effects assessment. *The contents of this abstract do not necessarily constitute or reflect US EPA policy.*

Interpreting in vivo effects of thyroid synthesis inhibitors through the lens of *in vitro* and *ex vivo* assays (PL)

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The US EPA has been charged to evaluate chemicals for their ability to disrupt endocrine pathways including estrogen, androgen, and thyroid hormone. Amphibian metamorphosis, which is regulated by thyroid hormone, is an ideal model system for investigating disruption of the thyroid-axis, and an in vivo metamorphosis assay has been developed for this purpose. In vivo tests that take several weeks to complete, however, are not an efficient or realistic mechanism for obtaining data on the full inventory of chemicals that are of concern to regulatory agencies. Advances in scientific knowledge and techniques at the molecular level of gene and protein expression provide promise for developing biomarkers using shorter term assays. Understanding the linkages between these early molecular events and organismal-level adverse effects is essential for ultimately utilizing short term assays as diagnostic tools. To this end, diagnostic markers of effects on thyroid hormone synthesis in the *Xenopus laevis* tadpole were developed using the model thyroid hormone synthesis inhibitors methimazole, propylthiouracil, and perchlorate. The effects of these chemicals were determined for inhibition of thyroid hormone synthesis in vitro, and on thyroid gland gene expression, and thyroid hormone synthesis and release in a thyroid gland explant culture system. These assays provided information on the proximal effects of the chemicals independent of the compensatory mechanisms in the developing organism. The insights gained regarding dose, time, and compensatory mechanisms for interpretation of in vivo gene expression, thyroid hormone measurements, and developmental sensitivity will be presented. This abstract does not necessarily reflect EPA policy.

METAL, COAL, AND DIAMOND MINING

A holistic approach to understanding selenium distribution, concentration, bioavailability and transfer through trophic levels in a northern aquatic environment. (PL)

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The Key Lake system in Northern Saskatchewan has undergone several years of low level releases of Se from metal ore milling. However, the transfer and factors affecting selenium distribution among environmental compartments are not well understood. Selenium accumulates in sediments and lower trophic level organisms. Therefore, seemingly innocuous water-borne concentrations can result in high selenium concentrations in sediments, and even higher concentrations in benthic macro-invertebrates. However, the effect of selenium on the reproduction and survival of fish is unclear. The route and magnitude of accumulation cannot be predicted using water-borne total Se concentrations alone. Instead, the bioavailability and transfer of selenium among environmental compartments of the ecosystem must be understood. Selenium species may also have different uptake kinetics and toxicological effects in different organisms (fish, benthic macro-invertebrates). This integrated study investigates the distribution, speciation, uptake and toxicology of selenium in a northern aquatic ecosystem, where results from prior studies elsewhere may not be sufficiently applicable. Program components over the next four years include field surveys, field mesocosm experiments and caging studies, involving all trophic levels (microbial, algal, invertebrates and fish).

Selenium investigations in the Elk Valley (British Columbia) and the Cheviot and Luscar coal mines (Alberta) (PL)

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Selenium (Se) concentrations are increasing in water downstream of the coal mines. However, in the Elk River Valley Se concentrations in benthic invertebrates, fish muscle and bird eggs, although elevated, have not increased over the last few years. The viability and productivity of fish and water bird populations do not appear to be adversely impacted; however, potential Se effects to trout in Alberta are being further investigated, and efforts continue to establish a definitive adverse effects threshold for trout in the Elk River Valley. It does not appear that human health or terrestrial wildlife (*i.e.*, ungulates) is being adversely affected. The primary focus is on monitoring and management. Management investigations include: predicting future Se releases under different mining scenarios and management approaches; determining factors affecting the cycling and conversion of inorganic Se once it enters the aquatic environment; and integrating present and future information to effectively manage Se releases. Research is also being conducted into treatment alternatives (passive bioreactor and in situ). Lentic and lotic areas are being mapped in the Elk River Valley to determine relative proportions of these habitat types both related to future Se studies, and to provide the basis for evaluating the regional significance of any localized aquatic impacts that may occur. Recommendations from a 2005 Se Science Panel have been applied to both monitoring and management; new studies include a Standard Operating Procedure for fish deformity analysis and predictive modeling of trout populations.

Physiological and biochemical responses of rainbow trout and brook trout exposed to elevated selenium from coal mines (PL)

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Selenium (Se), an essential element, bioaccumulates and can be toxic at levels slightly greater than those required for homeostasis. In areas with high Se in the parent rock, anthropogenic activities can increase Se in aquatic systems. Selenium causes reproductive deformities in rainbow trout (RT), at lower concentrations than for brook trout (BT). Understanding species-specific vulnerability to Se may explain why BT appear to out-compete Athabasca RT in Se contaminated streams. In this project, juvenile hatchery-reared RT and BT were stocked into two reference and two Se contaminated end pit lakes on coal mines in north eastern Alberta. Fish were sampled at 0, 6, and 12 months after stocking, and will be sampled at 18 and 24 months. Water Se levels were elevated in contaminated lakes, and selenite, the more toxic form of Se, was the highest in Pit C4. Liver glutathione levels were similar in the two fish species, and lipid peroxidation levels (cellular damage) were beginning to increase in fish from the contaminated lakes. At 6 months, liver glycogen levels were higher in the BT than the RT, although no site specific patterns were evident. RT were in better condition than BT at all sites except Pit C4. Additionally, BT from Pit C4 had a reduced ability to secrete cortisol suggesting the elevated selenite levels may adversely affect fish. Selenium accumulation and results from fish sampled at 12 months will also be discussed. Support: NSERC's MITHE-SN, Alberta Ingenuity, ACA, Allison Creek Brood Trout Station, Sam Livingstone Fish Hatchery.

Selenium accumulation and reproduction in breeding birds along the Key Lake uranium mine drainage in northern Saskatchewan (PL)

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Tree swallows nesting along the Key Lake mine drainage were monitored from 2007 - 2008 to discern whether elevated selenium concentrations in eggs were affecting reproductive success and productivity. Eggs from waterfowl and common loons were collected opportunistically and analyzed for selenium and possible abnormalities in developing embryos. In general, selenium concentrations measured in bird eggs from exposed areas were significantly greater than eggs from reference areas and in some cases (e.g., common loons) were sufficiently high to suggest the potential for adverse reproductive effects (using a threshold of 15μ g$\cdot$g^{-1}dw selenium in eggs). In 2007, selenium concentrations in tree swallow eggs reached a maximum of 13.3 μg$\cdot$g^{-1} dw at Wolf Lake, the point of effluent discharge, and also exhibited a gradient of selenium exposure related to distance along the mine drainage, indicating good site fidelity and localized selenium exposure. Assessment of tree swallow hatching success and productivity in 2007 showed similar hatching success and productivity for birds breeding at exposure areas relative to the reference area; however, hatching success was significantly reduced in an exposed marsh downstream of the mine. Despite this, no selenium-related deformities were noted in chicks or unhatched embryos, and unhatched eggs did not exhibit elevated selenium concentrations relative to other egg samples or selenium effects thresholds. Overall, initial findings do not indicate obvious adverse effects of selenium on tree swallows breeding downstream of the mill. Data collected during the 2008 field program will also be discussed.

Bioavailable versus total nickel (PL)

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A recent Ontario Court of Justice decision has the potential to change how government regulators interpret and enforce reported metal concentrations previously thought to have the capacity to impair water quality. The case involved charges by Ontario's Ministry of Environment (MOE) that Inco Limited ("Inco") "did commit the offence of discharging untreated mine effluent to Grassy Creek, which may impair the quality of the water..." and further, that Inco failed to properly notify MOE, contrary to the Ontario Water Resources Act (OWRA) (sn 30(1) and 30(2) respectively). The central focus of the case was the difference between total nickel and bioavailable nickel in terms of real or potential environmental effects. As environmental scientists we have known for decades that total metal results from water samples tell us practically nothing about the potential for toxic or other impacts on organisms. Despite this, the federal government and many provincial environment departments do not distinguish between total and bioavailable metals, as they continue to evaluate total metal concentrations to the Canadian Water Quality Guidelines or, in Ontario, to the Provincial Water Quality Objectives (PWQO). This presentation will discuss the metal objectives/guidelines, which are based on aquarium studies involving various species of fish and other organisms being exposed to different concentrations of dissolved, bioavailable nickel, copper and other metal salts. The observed toxic effects were then quantified against the concentrations of metals, safety factors were applied, and the guidelines and objectives were set. The presentation will comment on the physical and chemical toxicity-modifying factors that must be considered in evaluating impairment, and on the bigger problem of the ongoing practice of requiring that effluents and surface waters be tested for total metals - which will then be compared to the PWQOs and CWQGs and are guidelines for dissolved, bioavailable metals. The presentation will also discuss the application of the environmental sciences in legal cases, and the role of science in the "reasonable doubt" that complainants might be left with when apples are compared to oranges in the courtroom.

Development of a site-specific water quality objective for sulphate at the Highland Valley Copper Mine (PL)

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A site-specific water quality objective (SSWQO) for sulphate was derived for Pukaist Creek, which drains the Highland Valley Copper mine site. The approach taken in deriving the SSWQO involved initially re-evaluating the guideline using a Recalculation Approach, on the basis of new data for toxicity of sulphate to the aquatic moss, *Fontinalis antipyretica*. This species provided the most sensitive data in the current guideline derivation; however, the historical test was conducted using potassium sulphate, leading to questions about the contribution of potassium to toxicity. A Water Effects Ratio Approach was then used to establish a SSWQO for this anion, based on a comparison of toxicity tests conducted in laboratory control water with tests conducted in water from Pukaist Creek using three species that are sensitive to sulphate (*F. antipyretica, Hyalella azteca* and *Daphnia magna*). The results indicate that a defensible SSWQO could be established more than an order of magnitude greater than the generic guideline, largely as a result of the protective effect of higher hardness on sulphate toxicity. These results also suggest that inclusion of a hardness-based function into a water quality guideline for sulphate as a toxicity-modifying factor may be appropriate.

Effects of acid rock drainage discharging in nearshore groundwater on the marine intertidal community of Howe Sound, British Columbia (PL)

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The Britannia Mine operated primarily as a copper and zinc mine from 1904 until its permanent closure in 1974. Some 40 million tones of tailings are estimated to have been deposited mostly in the marine, subtidal slope and foreshore in the community of Britannia Beach, British Columbia. In addition to these reactive tailings, soil contamination in the backshore area contributes to low pH, metal-rich groundwater which discharges to the Howe Sound shoreline. Since 2004, an extensive monitoring program has been in progress as part of the Britannia Remediation Project. At the shoreline, we have measured copper in the shallow (~ 0.5 – 0.75 m), nearshore groundwater at concentrations up to 9 $mg \cdot L^{-1}$. We have observed alterations to the structure of the intertidal community and have concluded that the intertidal species distribution is closely related to the copper concentrations in nearshore groundwater. For example, *Cladophora* and *Enteromorpha* are prevalent in areas where nearshore groundwater copper concentrations are high. Conversely, *Fucus* and *Balanus* are virtually absent in these areas though present in adjacent and reference areas where nearshore groundwater copper concentrations are not elevated. Nearshore groundwater chemical composition appears to be influencing the adjacent marine surface waters in the intertidal zone; the pattern of metal contamination in the nearshore groundwater resembles that found in the adjacent marine surface water. Toxicity tests on groundwater and *in situ* caged mussel studies provide further support to conclude that recovery of the shoreline community is unlikely unless groundwater flux to the marine environment is controlled. However, it is not known if groundwater control alone will be sufficient as the shoreline substrate is comprised of reactive materials.

White sturgeon growth, morphology, and survival after exposure to Columbia River surface water at two sites in British Columbia, Canada (PL)

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The subpopulation of white sturgeon (*Acipenser transmontanus*) that resides in the Columbia River between the Hugh L. Keenleyside dam in British Columbia, Canada and the Grand Coulee dam in Washington State, USA has suffered nearly 30 consecutive years of poor recruitment. Factors such as altered flow regime due to damming, loss of critical habitat, predation, and pollution have been suggested as causes for the lack of recruitment, but none has been convincingly linked with the disappearance of young-of-the-year sturgeon. In the current study, surface water toxicity up- and downstream of a large metal smelter was examined as a possible contributor to the life-stage specific bottleneck in the white sturgeon population. Hatchery fertilized eggs from wild brood stock were exposed to Columbia River surface water from 1 d post-fertilization to 70 d post-hatch at two sites, one upstream and one downstream from the smelter effluent outflows. A filtered water control group was also examined to characterize any effects of inputs upstream of the study area not related to the smelter. The exposures took place in mobile laboratories outfitted with flow-through exposure chambers that allowed the white sturgeon to be exposed to the river water in real-time, a close representation of the natural exposure scenario. Concentrations of 18 metals in the water and growth, morphology, and survival of the larval sturgeon were examined over the course of the experiment. Histological evaluation of the larvae is also ongoing.

The Sherridon orphan mine site reclamation project (PL)

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The Sherridon Orphan Mine Site is a large abandoned mine site in northwestern Manitoba. The site is the responsibility of the Province of Manitoba and has been identified as a priority for the remediation of environmental and human health and safety risk associated with the acid generating tailings and waste rock on the site. Wardrop Engineering Inc. in collaboration SENES Consultants and Gartner Lee Limited developed the reclamation concept for the site and are now working on detailed design and implementation. Wardrop is utilizing the EEM guidelines in designing the post rehabilitation aquatic monitoring program to evaluate the effectiveness of the project. Reclamation work began in 2007 and is scheduled for completion in 2012. This presentation will overview the reclamation concept for the site and provides a progress report on implementation.

Uptake and depuration of environmentally relevant selenium species in the benthic macroinvertebrate *Chironomus tentans* (PO)

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Benthic invertebrates form an important link in the food chain transfer of selenium (Se) from the abiotic environment to higher trophic level organisms such as fish, yet little is known about the accumulation of Se in benthic invertebrates from the aquatic environment. In this study, a series of laboratory experiments were conducted to determine the rate and degree to which *Chironomus tentans* accumulates and depurates Se depending on the Se species present in water (selenate, selenite, selenomethionine). For each Se species tested, larvae were exposed to Se spiked culture water ($5\ \mu g\ Se{\cdot}L^{-1}$) for a period of 10 days. After the 10-day uptake phase, larvae were transferred to clean water for an additional 10 days to assess the rate of depuration. At specific time intervals during the uptake and depuration phases, larvae were collected and analyzed for whole-body Se concentration over time. Concurrently, larvae from selected replicates were exposed to Se spiked culture water until they emerged as adult insects. This allowed us to assess whether total Se tissue concentrations differ between the larval and adult life stages. Selenium speciation analysis of larvae (using synchrotron X-ray absorption spectroscopy) was performed on replicates collected at the end of the uptake and depuration phases for each Se species tested. Characterizing the uptake and depuration kinetics for *Chironomus tentans* exposed to environmentally relevant Se species will help assess the risk of exposure to fish feeding on benthic invertebrates such as chironomids.

Physiological effects of dietary, aqueous and in ovo selenium exposure in zebrafish (PO)

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Aquatic organisms especially fish are highly vulnerable to developmental selenium (Se) toxicity. Zebrafish is a model organism for toxicological research but little work has explored the toxicity of Se in this species. The objective of present study was to investigate physiological effects of Se exposures in zebrafish. Adult zebrafish were exposed to selenite and selenate (1:1 ratio) through water for 21 days and selenomethionine (SEM) ($10\ \mu g{\cdot}L^{-1}$) in food for 28 days. After exposure, samples were collected for trace metal analysis and adult fish were subjected to swimming performance analysis (critical swimming speed / U_{crit}). Se- exposed fish were bred to study maternal transfer of Se, analysis of frequencies of deformities in larval fish and long term fate of maternal transfer of Se in juvenile fish by measuring a stress biomarker (cortisol) and stored energy (glycogen and triglyceride) levels. Subsequently, the role of SEM in development of deformities in larval fish was studied. Fertilized eggs were injected with different concentrations (1, 3, 10, 30 ng/egg) of SEM by microinjection to determine a dose- response relationship for the development of deformities in larval fish. Future studies will focus on long term effects of SEM- induced subtle deformities in juvenile zebrafish.

Evaluating selenium uptake and toxicity in small-bodied fish downstream of a uranium milling operation (PO)

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Recent studies suggest that elevated selenium (Se) levels in the aquatic environment may cause chronic toxicity to fish and impact native fish populations. Exposure to selenium rich waters has been shown to lead to selenium bioaccumulation in fish, causing deformities and reduced survival in fry. This research aims to evaluate the importance of the feeding pathway with respect to Se uptake and speciation in wild populations of small-bodied fish. During the summer of 2008, spottail shiner (*Notropis hudsonius*) and lake chub (*Couesius plumbeus*) placed in separate cages sunk into the lake bottom were used to conduct 21-day feeding studies in lakes located downstream of a uranium processing mill in northern Saskatchewan, Canada. Two exposure lakes located downstream of the milling operation, and one reference lake situated in an adjacent watershed, were selected to investigate a gradient of Se exposure. Initial results indicated that spottail shiner deployed in both exposure lakes and the reference lake showed a decrease in condition factor after 21 days and may potentially be unsuitable for this type of study. In contrast, results for lake chub showed higher survival and condition factor at the completion of the 21-day trial. Individuals of both fish species will be analysed using synchrotron technology to quantify selenium uptake and speciation. This study also aims to target the specific organs and tissues where Se may preferentially accumulate in fish. The results of this study may be used to help create sustainable solutions for mining operations that discharge selenium rich effluent.

An evaluation of benthic invertebrate communities as an indicator of stream ecosystem health below active coal mines in the Elk River watershed (PO)

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The objective of this research was to determine whether the benthic invertebrate community structure is impacted by coal mining within the Elk River Basin, where open pit coal mining has occurred for more than 30 years. Benthic invertebrate communities assessed within Michel Creek below active coal mining were found to be within the natural variability as determined by the reference sites sampled, except for a decrease in sensitive species. The abundance and proportion of EPT and Ephemeroptera significantly decreased below coal mining, while Dipterans, particularly Chironomidae, increased. The changes in benthic communities suggest an impact to the aquatic health. Obvious changes in water quality below the coal mines included increased selenium, sulphate, nitrate and nitrite concentrations. Selenium concentration was the only water quality variable that showed a relatively strong negative correlation with changes in benthic community structure, while depth and predominant substrate were also strongly correlated.

An assessment of selected metal mining facilities across Canada using a benchmark based water quality index (PO)

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Traditionally, the assessment of water quality involves the statistical analysis of large and often complex datasets using a variety of statistical techniques. Unfortunately, such approaches are often demanding and subject to individual biases, limiting their widespread use and interpretation by resource managers and the general public. As a result, benchmark based water quality indices have been developed to quickly summarize and quantify overall water quality conditions based on guidelines for the protection of various water uses (e.g., drinking water, freshwater and marine aquatic life, etc.). Using a modified version of the Canadian Council of Ministers of the Environment's (CCME) Water Quality Index (WQI), we examine 16 metal mines regulated under the auspices of the National Environmental Effects Monitoring program. We utilize two benchmarks when calculating the WQI: 1) Numerical concentrations based on the CCME water quality guidelines for the protection of freshwater aquatic life; and 2) site-specific water quality objectives based on the 90th percentile concentration of each mine's reference condition. Index scores between undeveloped reference and exposure sites at each mine were compared to produce a relative percent difference score, allowing for a direct comparison

between approaches. WQI results based on CCME guidelines were best used to effectively monitor the absolute quality of water upstream and downstream of mine effluent discharges for the protection of freshwater aquatic life; whereas, the 90th percentile site-specific approach allows for a greater understanding of the relative changes in water quality between reference and exposure sites. Used concurrently, calculating the WQI using both approaches yield valuable information on the impacts of metal mining operations on freshwater aquatic life and on water quality conditions.

Determining the particle association of selenium in lake sediments using particle size separations and micro X-ray fluorescence imaging (PO)

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The Key Lake mine site in Northern Saskatchewan releases mine treated effluent into a nearby drainage system. The effluent contains low levels of selenium in the water, but selenium has built up in downstream sediments over time. This pool of selenium can subsequently be accumulated into biota, in particular benthic macro-invertebrates, and higher links in the food chain. Preliminary work has shown that selenium concentrations are well correlated to sediment total organic carbon. However, selenium speciation measurements of bulk sediment samples, made using X-ray absorption spectroscopy show a mixture of organic and inorganic selenium species in these sediments. The precise association of selenium in sediments improves our understanding if biogeochemical processes in the sediment, and thus our understanding of Se bioavailability. Here we present association of selenium as determined by particle size and detrital separations combined with micro X-ray fluorescence imaging, which allows us to image sediments in order to determine spatial co-occurrence of selenium with other elements.

Relationships between selenium speciation and microbial communities in depth profiles of lake sediments collected downstream of a metal mine effluent discharge (PO)

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The effluent generated and released by the Key Lake mine and mill operation in northern Saskatchewan, Canada, contains selenium in the chemical form of selenate. However, previous selenium speciation measurements indicate that red elemental selenium could be present in the lake sediments downstream of the effluent discharge point. It is therefore possible that microbes are partly responsible for changing the selenium speciation from oxidized, highly bioavailable selenate, to immobile, non-bioavailable red elemental selenium precipitates via a reduction reaction. Therefore, the microbial community structure at a specific location may be related to, and could provide information about, the speciation of selenium that is present in the sediment. Depth will be investigated as a dimension in the determination of selenium speciation and concentrations below the sediment-water interface. Preliminary work on the use of phospholipid fatty acids as a measure of microbial community structure along a known selenium concentration gradient in the Key Lake effluent drainage system will be shown. This includes depth profiles of selenium speciation in sediments and pore water selenium concentrations. In the future, fatty acid profiles will potentially reveal the relationship between whole sediment selenium concentrations and microbial community structure, and between sediment selenium speciation and microbial community structure.

MECHANISTIC ASPECTS OF METAL TOXICITY

Impact of Zn-spiked sediments on four benthic invertebrates: Implications for sediment quality guidelines (PL)

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Four aquatic invertebrates, the amphipod *Hyalella azteca*, the midge *Chironomus riparius*, the mayfly *Hexagenia spp.* and the oligocheates worm *Tubifex tubifex*, were exposed to different sediments from Lake Erie (hard water lake) and Lake Restoule (soft water shield lake) which had been spiked with Zn. Sediment spiking methods are discussed. The relationship between biological effects (survival, growth and reproduction) and sediment, water and body concentrations are compared for each organism. In addition, the impact of hard water (Lake Ontario) and soft water (10 % Lake Ontario) on the release and bioavailability of Zn from Restoule sediments to *Hyalella azteca* is examined. The changes in Zn bioavailability in relation to different sediment types as well as different overlay water types are discussed and the implications to Canadian sediment quality guidelines are examined.

Delineating the routes of uranium uptake and accumulation in freshwater midge *(Chironomus tentans)* larvae (PL)

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Delineating and quantifying the routes of metal uptake in freshwater invertebrates are important for understanding metal bioaccumulation and toxicity. However, the dietary route of metal accumulation and toxicity to freshwater invertebrates has, until recently, largely been overlooked. Some researchers have assumed aqueous exposure to be the major route of uptake, while others have suggested that the dietary route can be a significant contributor to total bioaccumulation of metals in benthic invertebrates. The uranium (U) mining and milling industry in northern Saskatchewan, Canada, can lead to localized increases of U in aquatic systems due to the release of effluent. We investigated the significance of dietary bioaccumulation of U in *Chironomus tentans* larvae fed Nutrafin® fish food prepared to contain nominal concentrations of either 100 or 1500 mg $U \cdot kg^{-1}$d.w. The experiment was designed as a 10-d test, with complete water and substrate renewal every 48 h to keep the aqueous concentration of U at minimum levels. Test endpoints included larval growth and U bioaccumulation. Since low levels of U were found in the exposure water (desorption from the food), the aqueous contribution to total U bioaccumulation was investigated separately using a water-only study with a similar test design, and aqueous U concentrations based on those measured in the dietary experiment (1.8 and 18.0 $\mu g \cdot L^{-1}$). Dietary U bioaccumulation resulted in growth inhibition in animals fed 1500 mg $U \cdot kg^{-1}$. No growth effects were observed from aqueous exposure to 1.8 and 18.0 $\mu g\ U \cdot L^{-1}$. The dietary U contribution was calculated from the total bioaccumulation in the dietary exposure study minus the aqueous contribution.

Physiology of dietary iron absorption in freshwater fish: implications for the absorption of other divalent metals (PL)

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Iron is an essential micronutrient for fish. Recent molecular evidences have indicated the presence of a specific iron transporter in fish known as the divalent metal transporter (DMT1–Fe^{2+}/H^{+} symporter). In mammalian systems, DMT1 is known to transport several other divalent metals in addition to iron. Despite the fact that diet is the major route of iron acquisition in fish, the physiological characteristics of dietary iron absorption and its possible interactions with other divalent metals are poorly understood. In the present study, we investigated the mechanism of intestinal iron transport in rainbow trout (*Oncorhynchus mykiss*) using an *in vitro* gut sac technique. Fe^{2+} and Fe^{3+} uptake were comparable at low luminal Fe concentrations (0.5 – 15 μM), whereas the bioavailability of Fe^{2+} was significantly greater than that of Fe^{3+} at high iron concentration (>15 μM). In addition, iron uptake was significantly reduced when luminal pH was increased from 7.4 to 8.2, thereby suggesting that the iron absorption pathway is proton-coupled. Interestingly, we found that the non-essential divalent metals lead and cadmium significantly inhibited iron uptake in all the intestinal segments (anterior, mid and posterior intestine). Iron uptake was also significantly inhibited by the essential divalent metals zinc, copper and nickel, although the competition profile varied spatially. Overall, our results suggested that the iron absorption in piscine intestine occurs predominantly *via* DMT1. Zinc, copper, nickel, lead and cadmium likely share a common absorption pathway with iron in piscine intestine, which may have important nutritional and toxicological implications for freshwater fish.

Selenium causes cytotoxicity in rainbow trout *(Oncorhynchus mykiss)* hepatocytes by oxidative stress (PL)

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Selenium (Se) contamination in the natural water is a growing concern in western Canada. Several *in vivo* studies have shown that Se exerts hormetic response in freshwater fish – acts as an essential micronutrient at lower concentrations and induces potential toxic effects beyond the threshold level. Liver is known to be the major site of Se accumulation and metabolism in fish. Se is known to induce oxidative damage in mammalian systems. We also hypothesized that the mechanism of Se cytotoxicity may be similar in piscine system. To test our hypothesis, we used isolated hepatocytes of rainbow trout in primary culture as a model in vitro experimental system. Trout hepatocytes were exposed for 24 h to 0, 50, 100 and 200 μM of Se as sodium selenite, the most toxic inorganic form of Se. Following the exposure, responses of several oxidative stress biomarkers were evaluated. We observed a dose-dependent induction of catalase and superoxide dismutase activities up to 100 μM. However, a significant increase in glutathione peroxidise activity was recorded at ≥100 μM exposure dosage. The reducing capacity of the cell, as determined by reduced to oxidized glutathione ratio, decreased with the increasing Se concentrations. A significant increase in membrane lipid peroxidation at the highest exposure dose was also recorded. A concomitant increase in the intracellular reactive oxygen species generation was also recorded with increasing Se concentration following an exposure period of 2 h. Overall, our present data suggests that Se mediates its toxicity in trout hepatocytes by inducing oxidative stress.

The dual nature of metallothioneins in the metabolism of heavy metals and reactive oxygen species in aquatic organism (PO)

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The purpose of this study examined the dual function of metallothioneins (MT) in the mobilization of heavy metals and superoxide anion (O_2^-) scavenging in aquatic organisms. By using an O_2^- generating system, liberation of free zinc from native and zinc-saturated MT was observed *in vitro*. Addition of the O_2^- generation system and H_2O_2 readily increased the di- and trimeric forms of MT as determined by gel electrophoresis. The oxidation of MT was quenched by the addition of a thiol reducing agent such as dithiothreitol. To determine whether the proportion of oxidized MT could change in contaminated environments, metal contaminated *Mya arenaria* clams were collected from a harbour in the St-Lawrence estuary. The levels of labile zinc, superoxide dismutase (O_2^- scavenging enzyme), lipid peroxidation and the oxidized/metallic form MTs (determined from an adapted version of a thiol-based assay for MT) were determined in the digestive gland. The results revealed that the induction of total MT levels was the result of increased oxidized MT at the expense of the reduced or metallic form of MT. Both SOD and labile zinc levels were induced and they were significantly correlated with the oxidized form of MT but not the metallic form of MT. We concluded that total MT level was related to zinc mobility and the activation of antioxidant mechanism such as SOD and followed the levels of oxidized MT. The metallic form of MT was negatively associated with zinc mobility but was associated with oxidative damage such as LPO. Overall, the oxidized fraction of MT appeared more closely related to detoxication while the metallic-form of MT was associated to metal toxicity via oxidative damage. The protective effect of MT during heavy metal contaminants will depend on the availability of metals and its capacity to sequester reactive oxygen species.

GENOMICS, PROTEOMICS AND METABOLOMICS IN AQUATIC ECOTOXICOLOGY

`Omics 2007: a way forward for `Omics science in Environment Canada's Water Science & Technology Directorate (PL)

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The `Omics 2007: genomics tools for aquatic environmental science workshop explored the potential of `Omics technologies and science to enhance Environment Canada's (EC) understanding of aquatic ecosystems and the effects of stressors. Improved understanding can strengthen EC's ability to conserve and protect the beneficial services provided by aquatic ecosystems. The workshop was the second in a series. The first, held in 2002, identified several areas that were likely to be enhanced by advances in genetics and molecular biology: environmental toxicology, pathogens in the environment, biodiversity, the impact of genetically modified organisms, analytical chemistry, and bioremediation. The intervening period has seen much progress. A workshop highlight was the fine panel of expert speakers from many areas of genomic science. `Omics 2007 played an important role in elucidating and confirming the key role that genomics is poised to play in support of EC's Science Plan in crucial areas such as understanding and managing the risks due to chemical stressors, organisms of concern, and microbial stressors, and developing tools that are predictive of higher level impacts and risks. We shall present an outline strategy, which was a product of the workshop, for furthering the development & application of 'Omics Tools in WS&TD's research and monitoring programs.

Differential gene expression in rainbow trout (*Oncorhynchus mykiss*) exposed to carbamazepine (PL)

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The antiepileptic drug Carbamazepine (CBZ) is difficult to remove during waste water treatment and as a consequence is present and persists in the aquatic environment. We used the Fluorescent RNA Arbitrarily Primed Polymerase Chain Reaction technique (FRAP-PCR) to identify rainbow trout genes that were differentially expressed after exposure to CBZ. FRAP-PCR revealed several gene transcripts from the livers and brains of rainbow trout that were up or down regulated by CBZ. The parent genes of those transcripts were identified by BLAST analysis of the cloned and sequenced cDNA. The FRAP-PCR data indicated that CBZ altered the expression of genes associated with apoptosis, transcription regulation, ion regulation, and a lipoprotein receptor. The CBZ exposures were repeated using different carrier solvents (water, ethanol, and PEG 200) after which quantitative-PCR was used to validate the differential expression of the FRAP-PCR identified genes.

Gene expression and interaction analysis in haemic neoplasia in the mussel *Mytilus trossulus* (PL)

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Physiological endpoints in mussels have been used for many years in environmental monitoring programs to assess biological impacts of water quality. More recently, analysis of gene expression has garnered interest as an additional and complementary monitoring tool. Interpretation of gene expression results can be hampered by the limited number of sequenced and annotated genes and by missing linkages between molecular and physiological endpoints. Here, we report on gene expression patterns of isoforms of the p53 tumor suppressor family in relation to haemic neoplasia in the mussel *Mytilus trossulus*. Haemic neoplasia is a proliferative disease of the haemolymph, which can decimate *M. trossulus* populations and has been linked to environmental, transmissible and potentially genetic factors. The p53-mediated molecular networks are well understood for mammalian cancers and are thus providing a hypothetical blueprint for the interpretation of gene expression data in relation to haemic neoplasia in mussels. We show that p53 and an N-terminally truncated deltaN isoform are expressed at significantly higher levels in neoplastic when compared to normal haemocytes. For the first time, we present the sequence and expression data for a Mdm2-like transcript in invertebrates which, in mammals, tightly regulates p53 in a negative feedback loop. Preliminary results from yeast-two-hybrid assays indicate that this Mdm2 homologue can bind to p53, suggesting that molluscan Mdm2 can regulate p53 activity. In conclusion, the in-depth investigation of the invertebrate p53 molecular networks provides us with much needed insight for the interpretation and validation of high-throughput toxicogenomic approaches which are currently being developed for eco-toxicological research using bivalve bioindicator species.

Altered gene expression in the brain and ovaries of zebrafish exposed to the aromatase inhibitor fadrozole: microarray analysis for hypothesis generation (PL)

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A part of an overall program of research aimed at examining system-wide responses of the hypothalamic-pituitary-gonadal axis in fish to endocrine active chemicals acting through a variety of modes of action, we exposed zebrafish (Danio rerio) to the aromatase inhibitor fadrozole for 24, 48, or 96 h. Global transcriptional response in brain and ovary tissue was examined using a commercially available 22,000 gene oligonucleotide microarray. Exposure to fadrozole altered expression of approximately 1200 transcripts in ovary and 1000 transcripts in brain. Transcripts altered in brain were functionally linked to differentiation, development, DNA replication, and cell cycle. Additionally, multiple genes associated with the one carbon pool by folate pathway (KEGG 00670) were significantly up-regulated. Transcripts altered in ovary were functionally linked to cell-cell adhesion, extracellular matrix, vasculogenesis, and development. Based on the transcriptional changes observed, we hypothesized that exposure to fadrozole elicits neurodegenerative stress and that fish cope with that stress through proliferation of radial glial cells. Additionally, we hypothesize that gene expression changes in the ovary of fadrozole-exposed zebrafish reflect disruption of oocyte maturation and ovulation due to impaired vitellogenesis. The contents of this abstract do not necessarily constitute or reflect US EPA policy.

Proteomic profiling for biomarker discovery in the zebrafish gill (PL)

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Using high-throughput mass spectrometer-based methods (2D LC-ESI MS/MS) we have identified approximately 6172 proteins in the gill of the zebrafish, *Danio rerio*. Proteins were characterized according to their cellular process, molecular function, and subcellular location. Those relevant to expected physiological and metabolic processes in the gill were identified and provided excellent coverage of important biochemical pathways. Greater than 14 % (2573) of the peptides in the analysis were classified as hydrophobic (GRAVY index >0.5) and more than 250 integral membrane proteins were found without using specific enrichment strategies. Approximately 40 proteins involved in xenobiotic metabolism and a number of established toxicological biomarkers were present. The goal of this project was to establish a baseline proteome for ongoing physiological and toxicological studies. Quantifying specific changes in the zebrafish proteome will be a powerful tool in identifying new biomarkers for emerging toxicants and novel engineered compounds.

Investigating adaptive, compensatory, and toxic responses of fathead minnow (*Pimephales promelas*) exposed to 17-ethynylestradiol using NMR-based metabolite profiling (PL)

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Determining the impact(s) of exposure on fish and other aquatic organisms by endocrine disrupting compounds (EDCs) is critical for determining the risks that these chemicals pose. However, to accurately evaluate these risks, beyond simply measuring a "before and after exposure" snapshot, researchers must assess the ability of the exposed organisms to adapt or compensate for the presence of these compounds. Due to the large number of samples required to map this complex response profile, a robust molecular technique with low per-sample cost of analysis is desirable. Therefore, we have employed a metabolomics approach for studying these responses in small fish toxicity models (e.g., fathead minnow) using nuclear magnetic resonance (NMR) spectroscopy. This approach provides the ability to measure molecular responses in different tissue and biofluid types, both rapidly and inexpensively, making it ideal for this application. Using this approach, we have been able to observe apparent compensatory responses to the presence of 17-ethynylestradiol over the duration of an exposure. Furthermore, it appears that after the chemical has been removed from the water (i.e. during a depuration phase) that fish are able in some cases to return to a near pre-exposure state, providing evidence of partial recovery. These results demonstrate the potential of this approach for improving the assessment of risk(s) that various EDCs pose to sentinel small fish species. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Medaka: an *in vivo* model for molecular ecotoxicology (PL)

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The major challenge of ecotoxicology is to identify mechanisms of toxic action in organisms exposed to environmental contaminants. A cost-effective whole adult medaka system was developed for high throughput multi-tissue studies, which allows concomitant identification, localization and quantification of molecular changes in a single animal *in vivo*. The small sized medaka provides a variety of useful developmental and reproductive endpoints for lifelong and multi-generational experiments. The freshwater medaka *Oryzias latipes* and recently the seawater counterpart *Oryzias melastigma* have been established as vertebrate models for freshwater and marine ecotoxicology. In the whole adult medaka system, over 200 serial sagittal sections can be produced from a single fish for multiple mRNA (by in situ hybridization, ISH) and protein (by immunohistochemistry, IHC) analyses. We have successful localized and quantified (by stereology) a number of target genes e.g. the telomerase reverse transcriptase (*omTERT*) gene, Leptin (*omLp*) gene, gonadal aromatase (*olCYP19a*) genes for the studies of their cell/tissue-specific expression profiles in medaka exposed to different environmental stresses (e.g. hypoxia, EDCs). By using serial fish sections, expression profile of target gene (e.g. omTERT mRNA) could be correlated to its protein expression and further linked to their cellular functions (e.g. cell proliferation by PCNA and apoptosis by TUNEL). This system also allows significant histopathological alterations to be identified in multi-tissues. Overall, the whole medaka system can serve as a vertebrate model for assessing multiple in vivo molecular responses to stresses in the aquatic environment.

Variations in p53-like coding sequence correlate with occurrence of haemic neoplasia in *Mytilus trossulus* (PO)

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Bivalve mollusks have been used as indicator organisms for water quality monitoring since 1975. Metro Vancouver initiated a study to test mussels (*Mytilus* sp.) for receiving environment monitoring in Burrard Inlet. Mussels are susceptible to haemic neoplasia. This disease, in part, is due to environmental and/or anthropogenic stresses. Previous studies indicated the involvement of analogs of the p53 tumour suppressor protein family. We have detected variations in the p53-like cDNA sequence of *Mytilus trossulus* associated with the presence of neoplastic cells in mussel haemolymph. Direct sequencing of p53 family cDNA revealed several sites of SNPs. Three of these sites occurred with high frequencies and constitute synonymous substitutions. We found that all late leukemic animals (with more than 95 % of neoplastic cells in haemolymph) had the same sequence composition at these SNP sites. Animals with the intermediate amounts of the neoplastic and normal cells showed a correlation between their p53 cDNA sequence and the ratio of different cell types. As well, sequencing of the same sample with different primers gave us different sequence compositions, which indicates a) the expression of unknown p53 isoforms, and b) presence of more than one p53 gene copy. Our preliminary conclusion is that the ratio of the different cell types is associated with the expression of different p53 isoforms and these p53 isoforms are expressed from more than one gene copy.

Real time PCR for monitoring persistence of *Bacillus* strains on the Domestic Substance List in water microcosms (PO)

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Microbial biotechnology products (wild-type or genetically modified) are used for industrial production, bioremediation, bio-control, etc. After their commercial release, live cells may persist and disseminate in the environment. One component required by regulatory agencies is the development of methods for detecting the survival of live cells and the generation of persistence trend data in relevant environments. In this study, the fate of three *Bacillus subtilis* strains on the Domestic Substances List (DSL) was monitored after introduction into river water microcosms containing indigenous microbial communities. During incubation, some cells may lose viability but still carry DNA; DNA-based detection methods may overestimate the number of live cells. Propidium monoazide (PMA) was reported to be highly selective in penetrating only into dead bacterial cells with damaged membranes but not to live cells with intact cell membranes and walls. We tested and verified the effectiveness of PMA in binding DNA of dead cells in the *B. subtilis* strains. Subsequently, we used PMA to treat *Bacillus* cells sampled from river water microcosms. DNA of *Bacillus* cells was quantified by real time PCR with functionally specific amplified fragment length polymorphism (AFLP) primers. The results showed that live cells of the three *B. subtilis* strains can be detected from only a few days to one hundred days after introduction into the microcosms at 10^7 cells·mL^{-1}.

NORTHERN AND ARCTIC ECOSYSTEMS

Sediment quality in the Mackenzie River basin: metals (PL)

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Data collected for the Mackenzie Gas Project (MGP) and the Regional Aquatics Monitoring Program (RAMP) provide insight into the current spatial patterns of sediment quality in the Mackenzie River and its tributaries. The sediment samples were analyzed for a number of metals. Arsenic concentrations range from 0.1 to 24.7 mg·kg^{-1}, with an average value of 5.4 mg·kg^{-1}. Arsenic concentrations exceed the Interim Sediment Quality Guideline (ISQG) of 5.9 mg·kg^{-1} in 50 instances, in particular in the Sahtu region along the Mackenzie River and its confluences with the Great Bear River and the Mountain River, and in the Mackenzie River delta. Concentrations of As were generally lower in the Athabasca River basin, but did exceed 5.9 mg·kg^{-1} at many sites. Concentrations of As exceeded the probable effect level (PEL) of 17.0 mg·kg^{-1} at two sites in the Mackenzie River delta, and at one site in the Athabasca River basin, reaching 18.5 mg·kg^{-1} in Stanley Creek, a tributary to the Muskeg River. Concentrations of Cd ranged from 0.1 to 1.6 mg·kg^{-1}, with an average of 0.2 mg·kg^{-1}. The ISEQ guideline for Cd of 0.6 mg·kg^{-1} was exceeded at 11 sites, 8 of which, including Jimmy Lake, are located in the Mackenzie River delta. The other sites where the ISEQ guideline for Cd was exceeded were Prohibition Creek (1.6 mg·kg^{-1}) and an unnamed lake in the Sahtu region (0.6 mg·kg^{-1}). Concentrations of Cd were generally lower in the Athabasca River basin. Pb and Hg did not exceed ISQG. Based on the metal data, there is no evidence of impacts from oil sands development at this time. These observations, however, can be used to direct environmental monitoring as further development in the basin takes place.

Metals and organic contaminants in lake trout and burbot from Great Slave Lake, Northwest Territories; spatial patterns and time trends (PL)

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Great Slave Lake is one of three large lakes in the Mackenzie River Basin, and the major depositional basin for contaminants entering the lake from the Slave River. We have been monitoring contaminants in lake trout and burbot since the early 1990s as part of the Northern Contaminants Program assessing contaminant levels and trends in Arctic and subarctic ecosystems; the majority of contaminants are believed to enter such systems through long-range atmospheric transport. Fish are being caught from one (burbot) or two (lake trout) locations in Great Slave Lake under differing influences from the Slave River and analyzed for a variety of organic contaminants and metals. In addition, fish are measured, weighed, and age, sex, lipid, and carbon and nitrogen isotopes determined. Some organochlorine contaminants such as HCH have shown a decline in concentration over the study period while others such as PCBs have shown little change. Mercury concentrations are increasing. There is some evidence of declining lipid levels and changing feeding patterns in lake trout and increasing lipid levels in burbot which may be driving and/or modifying these contaminant trends. Some contaminant trends may be related to a warming trend for the study regions (e.g., mercury) while other trends may be in response to reduced contaminant use and an absolute reduction of these contaminants circulating in the environment (e.g., HCH, PFOS and PFSA). The influences of the Slave River on contaminant patterns and the biology of burbot and lake trout are briefly discussed.

Temporal trends and spatial variations in persistent organic pollutants and metals in sea-run char from the Canadian Arctic (PL)

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We are investigating metal and organic contaminant levels in sea-run char as part of a Northern Contaminants Program project designed to assess temporal trends in contaminants and to provide communities with information on the chemical levels in important country foods. We have analyzed fish from Paulatuk in the western Arctic, east to Pangnirtung on Baffin Island, and from Pond Inlet on the tip of Baffin Island, south to Labrador and Hudson Bay. Sea-run char are larger and older in our more northerly study locations, most likely due to a combination of lower temperature and fishing pressures, both of which affect longevity. Contaminant levels are very low in sea-run char throughout our study area, especially mercury. PCB, HCH and toxaphene concentrations have declined markedly at Pond Inlet with less dramatic declines in chlordane and DDT; CBz concentrations remain unchanged. There is little evidence of a long-term change in mercury concentrations in char at Cambridge Bay. We present data comparing measured and estimated mercury and PCB concentrations in sea-run char, landlocked char and seals to demonstrate the extremely low contaminant concentrations in sea-run char fillet. Finally, we compare chemical levels in sea-run char with those in lake trout from Great Slave Lake and demonstrate than the majority of contaminants occur in lower concentrations in sea-run char despite their similar ages and much higher lipid content than lake trout. It is possible that lake trout are exposed to greater and more accessible contaminant reservoirs than sea-run char; contaminants are more efficiently buried in sedimentary sinks in the marine environment.

Spatial comparison of mercury bioaccumulation in Arctic char lakes from 4 Canadian Arctic regions – why food webs matter (PL)

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Concentrations of mercury [Hg] have increased slowly in landlocked Arctic char over a 10-15 year period in the Arctic. Hg fluxes to sediments also show increases in most Arctic lakes. We hypothesized that climate warming might enhance Hg methylation and alter food chain biomagnification. Correlation of [Hg] with $\delta^{15}N$ was used to investigate [Hg] bioaccumulation in 20 lakes in Nunavut and Nunavik (Canada). Sites were selected along an N-S transect to compare different climatic regimes. Food web samples included water, sediment, periphyton, benthos, and Arctic char of varying size-classes. Samples were analysed for [Hg] by direct combustion (DMA 80), methylHg using CV AFS following GC separation, and bioaccumulation factors (BAF) were calculated. Stable isotopes were determined using isotope ratio MS. The $\delta^{15}N$ of char varied from 6.8-10.3 ‰ (Kent) to 9.5-14.9 ‰ (Cornwallis). Inter-lake variability of food web length of 3-6‰ was observed. Total mercury (THg) in char muscle tissue ranged from 0.05 – 1.8 $\mu g \cdot g^{-1}$ (Ellesmere), and 0.06 – 3.4 $\mu g \cdot g^{-1}$ (Cornwallis) to 0.03 – 0.5 $\mu g \cdot g^{-1}$ (Kent) by area. While variability of methylHg bioaccumulation was observed from lake to lake, with logBAF varying from 6.98 – 8.07, THg in water did not directly predict char muscle THg. Intra-lake variability of char THg was related to trophic position, which was used to adjust [Hg], making spatial comparison possible. Results indicate that food web length and trophic position are key factors in explaining spatial variability of concentrations of Hg in landlocked Arctic char. These factors are crucial to understanding possible linkages between increases in mercury tissue concentrations and climate warming.

OIL SANDS RESEARCH

Carbon dynamics, food web structure & reclamation strategies in Athabasca oil sands wetlands (CFRAW) - overview and progress (PL)

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Wetlands will make up 20-50 % of the final reclamation landscape of areas surface mined for oil sands in northeastern Alberta. CFRAW is a collaboration among 7 mining partners and 5 university labs to study effects of mine tailings and process waters on development, health and function of wetland communities expected to form in post-mining landscapes. Our work tests predictions about how quickly wetlands amended with reclamation materials approach the conditions seen in reference wetland systems. Supplementing constructed wetlands with stockpiled peat or topsoil is expected to accelerate succession and community development. The hydrocarbons in tailings (bitumen) and water (naphthenic acids) that occur in wetlands constructed with mine process materials are initially toxic, but may ultimately serve as a surrogate source of carbon once they degrade and/or are metabolized by bacteria. We are assessing the sources, biological uptake, pathways, and movement through the food web of materials used by the biota in constructed wetlands. Studies in progress are evaluating how productivity of new wetlands is maintained. We are monitoring net ecosystem productivity, rates of organic carbon accumulation from microbial, algal, and macrophyte production, and influx of outside materials. We are also comparing rates of leaf litter breakdown and microbial respiration to determine how constituents speed or slow food web processes of young and older wetlands. Stable isotope measurements of carbon and nitrogen in food web compartments indicate which sources are incorporated into the food web as wetlands age; how this influences community development, food web structure and complexity, and the productivity and health of fish, amphibians, and wetland birds. Flux estimates will be combined to determine whether wetlands built with peat amendments can be expected to maintain their productivity and have the potential to ultimately become true peat lands. The research will provide a conceptual model of carbon pathways and budgets to assess how the allocation of carbon among compartments changes as newly formed wetlands mature in the boreal system. Ultimately, we will recommend the materials and strategies most effective and economical in producing a functioning reclamation landscape.

Metal leaching from oil sands coke and associated characterization of leachate toxicity (PL)

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Thermal upgrading of oil sands bitumen in Alberta, Canada, produces an enormous volume of coke as a by-product. This large volume of coke may someday be integrated into reclamation landscapes. Under different environment conditions, the trace elements associated with this coke could therefore be released into the surrounding landscapes. In the present study, the leachability of metals from coke was examined over a 15 day period in a batch leaching process under two different pH conditions (i.e. 5.5 and 9.5). Samples of leachate from both pH treatments were analyzed for dissolved trace elements. At the end of the 15 day leaching period, toxicity of coke leachate was evaluated using a *Ceriodaphnia dubia* standard three-brood chronic test with survival and reproduction as endpoints. Results showed that manganese and nickel releases were significantly ($p \leq 0.05$) higher under pH 5.5 leaching condition when compared with pH 9.5 conditions. For aluminum and vanadium the opposite was true. Toxicity results of coke leachate revealed LC50 values to be 6 % and 28 % for pH 5.5 and 9.5 treatments, respectively. Further, the concentrations of vanadium and nickel in 100 % coke leachate from both pH treatments were well above the 7-d LC50 values of 550 $\mu g \cdot L^{-1}$ and 3.8 $\mu g \cdot L^{-1}$, respectively. Evidence gathered from chemical characterization of oil sands coke leachate suggests that nickel and/or vanadium could be the likely causes of leachate toxicity. Toxicity identification evaluations (TIEs) were subsequently used to further characterize the cause(s) of coke leachate toxicity.

In vitro evaluation of the toxic effects and endocrine disrupting potential of oil sands processed water and naphthenic acids (PL)

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There is increased scientific and public concern regarding the adverse effects of oil sands process-affected waters (OSPW) produced in the oil sands industry of Alberta. Naphthenic acids (NAs) had been identified as the primary toxic constituents of OSPW. While research into the toxic effects of OSPW and NAs continues to grow, very little is known regarding the endocrine modulating effects of OSPW and NAs. To this end we have initiated a series of in vitro studies to evaluate potential endocrine modulating effects of OSPW and their constituent NAs. The H295R steroidogenesis bioassay was employed to investigate the impact of OSPW and NA on steroidogenesis. Specifically, we performed dose-response and time course studies to evaluate the impact of OSPW and NAs on testosterone and estradiol production. Aromatase activity and transcript abundance of the key 11 steroidogenic enzymes were also quantified to complement analysis of hormone levels. The estrogenicity/anti-estrogenicity of OSPW and NAs was tested by the MVLN trans-activation assay. In vitro cell viability and apoptosis (live-dead) caused by OSPW and NAs was quantified by the MTS reduction and caspase-3/7 activity in H295R and MVLN cells. A discussion of the endocrine modulating potential of OSPW and NAs will be presented.

Biodegradation of complex naphthenic acid mixtures and a probable link between congener profiles and aquatic toxicity (PL)

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The aquatic toxicity of process affected waters (OSPWs) from the Athabasca Oil Sands (AOS) in northern Alberta, Canada, is at least partially related to a somewhat persistent group of dissolved organic acids known as naphthenic acids (NAs). In this research, we evaluated the potential for the biodegradation and associated reduction in aquatic toxicity of OSPWs using flow-through laboratory wetland microcosms, and identified changes in NA composition for a period of 52 weeks. Experimental manipulations included two types of OSPWs (Syncrude and Suncor), increased nutrients (nitrogen and phosphorus enrichment), and short and long hydraulic retention times (HRTs) (40 and 400 days). HPLC/QTOF analysis was used to track changes in NA mixture profiles (fingerprints) in each treatment over time. Based on first order kinetics, the biodegradation of NAs in Suncor OSPW was significantly faster than that in Syncrude OSPW ($p < 0.025$). The biodegradation of NAs in both sources of OSPW was enhanced under longer HRTs ($p < 0.005$), whereas the influence of nutrient addition was minimal. More rapid degradation was observed for NAs that had the lowest degrees of cyclization and lowest carbon number. This is consistent with previous trends observed for aerobic microbial degradation of NAs using laboratory incubations. Within the NA mixture fingerprint, we also identified the three most persistent fractions of NA homologues, the persistence of which may explain the lack of correlation between the predominantly unchanged toxicological response (EC20) as measured by Microtox® and the observed reduction in total NA concentration (up to 78 %) over a period of 52 weeks.

PAH sediment studies in Lake Athabasca and the Athabasca River ecosystem and the Mackenzie River ecosystem: natural sources and the impacts of oil sands development (PL)

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The Mackenzie River Basin is rich in hydrocarbon reserves providing a natural source for PAHs. The potential for increased loading is a concern as mining activity intensifies with the expansion of oil sands operations in the Fort McMurray area and with the proposed expansion of gas extraction in the Beaufort Sea, Mackenzie River delta and surrounding area. As a consequence of such activities, there is much interest in the presence of hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) in the environment, their sources, and the impact of industry in modifying concentrations in the major river and delta ecosystems. Here we report on our characterizations of PAH sources and composition in the major depositional basins (Mackenzie River delta, Great Slave Lake, Lake Athabasca, and Peace-Athabasca delta) and in the Mackenzie and Athabasca rivers and their tributaries. Natural petrogenic sources are numerous with the consequence that compounds of petrogenic origin dominate in tributary and downstream sediments. Combustion sources are of lesser importance in the major depositional areas although loadings of pyrogenic PAHs can be high following forest fires and may be expected to increase with climate change. In the Athabasca River basin, highest PAH concentrations are associated with locations near exposed bitumen beds and secondarily with fine-grained sediments in the tributaries and in downstream depositional areas. There are subtle differences in PAH composition in the three major depositional basins that reflect differences in sources and transportation distances. Concentrations of some low molecular weight PAHs exceed interim sediment quality guidelines. Temporal trends in PAH concentrations in sediment cores appear to be related to hydrological change with some suggestion of recent increases in low molecular weight PAHs. We conclude by addressing the potential anthropogenic sources for PAHS in Lake Athabasca, the Peace-Athabasca delta lakes, and Great Slave Lake.

Spatial and stress-related variation in benthic microbial respiration in northeastern Alberta wetlands (PL)

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The goal of this project is to study microbial production and sediment respiration in newly formed wetlands, as it relates to effects of oil sands process material (OSPM). Open pit oil sands extraction results in the loss of natural wetlands in the Fort McMurray area, which presents an opportunity to study ecosystem processes, including microbial respiration in reclamation sites contrasting in age and condition. Microbial communities are important in cycling nutrients, respiring carbon dioxide and methane and storing large fractions of carbon in biomass. I studied sediment microbial respiration (volume of gas/unit of microbial biomass/unit area/day) of 10 wetlands contrasting in age, and sediment characteristics. Sediment-produced gases were collected in summer 2008 using microcosms inserted into the substrate and analyzed using gas chromatography. Sediment microbial production was measured using leucine incorporation. Methane gas and CO_2 (estimated from evolved gas + increase in alkalinity) production were low and variable (mean±SE volume of $CO_2 = 12.2 \pm 27.0$ mL n=10 wetlands). Estimates of net gas and microbial production will be combined to estimate net benthic respiration, extrapolated to wetland annual fluxes. Respiration will be contrasted between wetlands of differing in status (OSPM-affected vs. reference). OSPM-affected bacterial communities are expected to respire more carbon than reference communities. This research will give a basic understanding of the fate of carbon in sediments of boreal wetlands and address spatial heterogeneity of intra-wetland carbon cycling. As well, estimates of wetland greenhouse gas flux will help inform whether current reclamation strategies will ultimately produce sustainable wetland habitats.

Health assessment of tree swallows (*Tachycineta bicolor*) on the oil sands using stress, immune function and growth indicators (PL)

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As oil sands mining continues to expand on the Athabasca oil sands of northern Alberta, companies are exploring reclamation strategies for tailings water and are evaluating the ecological effectiveness of constructed wetlands for the bioremediation of liquid and solid tailings contaminated with naphthenic acids, polycyclic aromatic hydrocarbons, and dissolved inorganic ions. Wild tree swallows (*Tachycineta bicolor*), nesting on experimental wetlands receiving tailings or water from tailings ponds, have been designated as upper trophic level biological sentinels. Examining health variables- including stress and immune function indicators- in tree swallows nesting on experimental wetlands, provides a measure of reclamation success and ecosystem health of the reclaimed sites. From May to July 2008, clutch size, egg mass, and hatching and fledging success of 40 breeding pairs of tree swallows was measured on three experimental wetlands on Suncor Energy Inc. and Syncrude Canada Ltd. oil sands leases. These wetlands, Shallow Wetlands/Demonstration Pond (Syncrude), Consolidated Tailings and Natural Wetlands (Suncor), represent sites of different ages and different categories of wetland reclamation; mature, maturing, and newly reclaimed. Due to lack of nesting success on the selected reference site, Shallow Wetlands/Demonstration Pond was chosen as the reference site as it has undergone nearly complete bioremediation and has an extremely low level of contamination. Nestling growth and survival were measured in 199 tree swallows nestlings on the three experimental wetlands. Two mid-weight nestlings from 12 nest boxes on each study site were examined for feather corticosterone levels, adaptive immune function using a unique application of the delayed-type hypersensitivity test which reflects an integrated immune response, and innate immune function of circulating white blood cells (phagocytes) using a novel, chemiluminescence assay.

Nucleolar organizer (NO) size as a measure of instantaneous growth in Chironomus riparius larvae (Diptera: Chironomidae): a tool for monitoring individual and population responses to stress (PL)

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Salivary glands of midge larvae possess giant polytene chromosomes. When genes on these chromosomes undergo transcription the normally condensed chromatid strands uncoil and are visible as puffs. The nucleolar organizer (NO), visible as an especially large puff, shrinks when a larva is subjected to physiological or toxicological stress. It is unknown, however, whether NO regression can predict an individual's developmental fate (survival or growth). In the laboratory, two experiments were conducted using *Chironomus riparius* larvae to relate NO size to chironomid growth. In one experiment, five treatments varied only in diet quality, which was manipulated by providing larvae with 1.0 mg of food/individual/day, but varying the ratio of Tetramin™ to non-nutritious methyl-cellulose (0:1, 1:7, 1:3, 1:1, 1:0). A second experiment followed a 2 x 2 factorial design. The factors were growth period (initial, days 0-7; final, days 8-14) and diet quality (low, 1:7; high, 1:0). Diet quality and growth period significantly affected individual biomass. NO size was related to the quality of the diet provided at the end of the experiment, regardless of larval biomass. Thus, NO size appears to be related to growth rate at time of collection rather than larval size. We propose using NO size of larvae in natural populations as a measure of growth on which to base estimates of secondary production and as a novel way to monitor individual and population responses to environmental stress. Preliminary field measurements of larval production and NO size from oil sands-affected and reference wetlands are consistent with laboratory findings.

Testate amoebae as indicators of ecosystem establishment in wetlands impacted by oil sands processed materials (OSPM) (PO)

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Oil sand mining in the Athabasca Basin in northeastern Alberta is a rapidly growing industry. As economics continue to drive expanding mining practices, waste management and reclamation strategies for wet landscapes are becoming increasingly important. Many types of wetlands have been created on oil sand leases with different OSPM and non-OSPM amendments in order to determine environmental sustainability. There is a critical need for indicators of wetland health to determine the viability of these systems. Microbial communities in created wetlands are exposed to high levels of bitumen and the associated components (NA's, sulphates and chlorides) present in oil sands. Studies have shown these communities have some capacity in breaking down hydrocarbons. The composition of microbial communities is being characterized within a range of wetlands using epifluorescence microscopy to determine the proportion of both fungi and bacteria. Light microscopy is being used to determine the proportion and identity of testate amoebae (protists). This project aims to determine the practicality of testate amoebae assemblages in wetland types as related to bacterial and fungal growth and indicate microbial community health, wetland establishment, and performance of created wetlands. This project will report the results of field work conducted in 2007 and 2008.

Sediment oxygen demand of wetlands in the oil sands region of north-eastern Alberta (PO)

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Sediment oxygen demand (SOD) is the rate at which dissolved oxygen is consumed by biochemical processes at the sediment-water interface. It is a major factor that contributes to oxygen depletion and will fluctuate depending on the sediment present. The reclaimed landscape in the Alberta oil sands mining area contains both reference and oil sands process-affected wetlands constructed using varying sediment compositions. These sediments, particularly those derived from oil sands process materials (OSPM), may alter the biochemical reactions that take place and ultimately affect SOD. We measured SOD in a suite of constructed wetlands varying in age, presence/absence of OSPM, and/or topsoil. Changes in oxygen concentration were measured using dissolved oxygen loggers inserted into dome-shaped chambers resting on the sediment. Complementary measurements of respiration (CO_2 elution) permitted us to quantify the biological sediment oxygen demand (BSOD) component of SOD. The chemical sediment oxygen demand (CSOD) was then determined by subtraction from SOD. We expect wetlands reclaimed using OSPM to have a lower BSOD: CSOD ratio than reference wetlands. Residual ammonia in OSPM sediments may react with sulphate and bind phosphorus. This reduces phosphorus bioavailability and may impede submergent macrophyte growth. Consequently, wetlands mostly affected by CSOD will have fewer submerged macrophytes than BSOD-dominant wetlands. This research will help clarify the role of SOD in wetland function and in the reclamation process.

Wetland plant community dynamic over time: a comparison between natural and created wetlands affected by oil sand mining (PO)

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Reclaimed wetlands may never recover their original function and integrity. Instead, they may be characterized by alternative ecosystem states or a succession different from the one observed in natural wetlands. The objective of this study is to assess the degree of success of wetland reclamation in landscapes cleared for oil sands mining in north eastern Alberta. I will compare plant community dynamic through time in both restored and natural wetlands. This study assesses possible differences in succession among wetlands developing on mineral substrates vs. those whose substrates have been covered ("amended") with a layer of oil sands mining process tailings and/or a layer of peat in a 2 x 2 factorial design (amended vs. unamended; tailings vs. peat). Changes in emergent macrophyte community attributes will be assessed through 1) transect sampling to contrast community composition in 40 created and natural wetlands of varying ages and amendment classes; and 2) A longitudinal study using annual aerial photos of natural and created wetlands to observe long-term trend changes. Plant community changes in time will be measured using eight elements of comparison: overall vegetation cover, species richness, dominance, density, biomass, growth rate, height, and flowering capacity of dominant species. The distance from potential seed sources, the absence of seed bank and water level variation are expected to direct created wetlands to states not observed in natural ecosystems. The chemistry of tailing constituents, as well as the absence of organic matter is expected to affect the dynamics of plant community in created wetlands.

Using biofilms and grazing chironomids (*Diptera: Chironomidae*) to determine primary production, nitrogen stable isotopic baseline and enrichment within wetlands differing in anthropogenic stressors and located in the Athabasca oil sands region of Alberta (PO)

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The effects of oil sands process materials (OSPM) and construction disturbances on primary production and nitrogen stable isotope enrichment were studied in reclaimed and reference wetlands situated on Athabasca oil sands mine sites. The succession and viability of reclaimed wetlands is assessed through productivity and food web analyses. Primary production is estimated through chlorophyll a (Chl a) concentrations and biomass. Carbon (C) and nitrogen (N) stable isotope ratios can reveal energy sources, storage and the magnitude and direction of energy transfer within food webs. Our objectives were to determine primary productivity, the N baseline, and N enrichment from biofilms and grazing invertebrates colonizing artificial substrates immersed in the water column of 2 OSPM-affected, 2 constructed reference and 2 natural reference wetlands. Algal biomass, (Chl a concentrations), ranged from $0.018\ \mu g \cdot cm^{-2}$ to $2.32\ \mu g \cdot cm^{-2}$. Biofilm biomass in OSPM affected and constructed wetlands was significantly lower ($p<0.05$) than in natural reference wetlands. Constructed wetlands were also lower in Chl *a* concentrations than non-constructed reference wetlands. We expect the N baseline and enrichment ratios, to be greater in OSPM affected than in reference wetlands due to incorporation of hydrocarbons and ammonia at the base as well as to the inefficiencies resulting from impaired metabolic processes exhibited by stressed consumers. The lower biomass and Chl *a* concentrations in OSPM-affected and constructed wetlands suggests that both anthropogenic disturbance and OSPM have a negative effect on primary productivity and therefore overall wetland function.

The effects of nutrient enrichment on oil sands reclaimed wetlands (PO)

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The extraction of bitumen from oil sands in Alberta, Canada, generates a large amount of processed material that must be reclaimed. Using processed material to create wetlands and shallow lakes is a challenge as this material has the potential to have a strong impact on aquatic faunal and floral colonization. In this case, the quality of the substrate may be poor due to physical (particle size, organic content) and/or chemical characteristics. Present in the processed materials are naphthenic acids, polycyclic aromatic hydrocarbons, and increased salinity, which pose toxic threats to the environment. One approach to improving the quality of the substrate used in reclamation is the addition of nutrients. Nutrient enrichment promotes primary productivity which settles out creating a biological layer to confine toxic processed material and provide a more favourable substrate for colonization. The objective of this study was to examine the influence of nutrient addition on primary production by chlorophyll *a* and total biomass analyses. Nutrients were added to microcosms to create differing levels of fertility (oligotrophic to hyper-eutrophic conditions) for each of three water types that vary in naphthenic acid concentration and for each of three different potential reclamation substrates (peat, sand, mature fine tailings (50 %) + sands (50 %)). Results showed different levels of growth depending on both the water and substrate type. Generally, combined planktonic and periphytic growth was highest in water with high levels of dissolved organic and inorganic carbon.

Rapid assessment of toxicity of oil sands process-affected waters using fish cell lines (PO)

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Large amounts of tailings and process-affected waters are associated with the extraction of petrochemicals from the oil sands of Athabasca, Alberta. The oil-sand's process-affected waters (OSPAW) are being evaluated in acute and chronic toxicity tests with various model organisms to evaluate the safety of reclamation ponds where OSPAW's are being deposited. Classical toxicity assays that evaluate the ability of effluents to induce death in tested organisms usually require large volumes of test waters, take time and are costly, and/or may involve expensive analytical assays to determine contaminants present. Cytotoxicity assays with indigenous and model fish cell lines could prove useful for rapid comparative toxicity evaluation of large number of samples retrieved at various temporal and spatial sites at a fraction of cost and time required to perform *in vivo* assays. In this study, we report on a rapid fluorometric assay using several fish cell lines to assess the acute toxicity of 20 water samples including OSPAW. The water samples were mixed with media salts and directly evaluated on cells in culture without prior extraction procedures. This evaluation was done in blind and a consistent pattern of toxicity was observed with the tested cell lines. This approach could alleviate or minimize the need of using whole organisms for toxicity assessment of OSPAW.

Relating zoobenthic & emergent terrestrial insect production to tree swallow (*Tachycineta bicolor*) nestling diet in oil sands wetlands (PO)

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Tree swallows readily inhabit constructed nest boxes and forage near their nest on flying insects of terrestrial and aquatic origin. Production of zoobenthos and emergent aquatic insects is, therefore, an integral link in the relationship between lower- and upper-trophic-level animals. The presence of oil sands process materials (OSPM) influences wetland macroinvertebrate community composition and production. We assessed the structure of wetland food webs as it relates to the transfer of production from aquatic sediments to nestling tree swallows in two reference and two oil sands affected wetlands. To estimate benthic invertebrate composition and production, we collected exuviae of emerging aquatic insects from floating traps (n=5/wetland) and flying insects from sticky traps (n=5/wetland) and aerial sweeps (n=3/wetland) every 3 days during the tree swallow nestling period (3 weeks in June/July). Tree swallow nest boxes, placed around the perimeter of 2 reference and 2 OSPM-affected wetlands in spring were monitored during egg laying and incubation. Diets of nestlings 10-14 days old were determined by placing a ligature around the neck of each nestling, preventing the passage of food into the esophagus for 45 min. Food boluses were collected from nestlings fed by the parents during that time. Oil sands-affected wetlands had lower aerial insect abundance. However, they represented over half of the 32 total boluses collected. This study will provide insight into whether oil sands-affected wetlands are ecologically viable and capable of supporting terrestrial predators that rely on export of zoobenthic production.

Are there toxic interactions between salinity and naphthenic acids in the toxicity of oil sands process water to freshwater invertebrates? (PO)

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The oil sands mining industry in the Athabasca River area, Alberta, uses large quantities of water during the oil extraction process. The resulting oil sands process water (OSPW) is proposed to be part of future reclamation landscapes so understanding its toxicity to freshwater invertebrates is important in order to develop environmentally acceptable OSPW reclamation plans. The OSPW typically contains elevated concentrations of salts and naphthenic acids (NAs), but low levels of other contaminants such as PAHs and metals. This project investigated the combined toxic effect of NAs and salinity on freshwater invertebrates. The toxicity of OSPW from selected water bodies was first determined using laboratory cultured *Ceriodaphnia dubia*. The pond waters that elicited a toxic response had elevated levels of NAs and salinity, but the concentrations of salinity ions varied greatly among ponds. Results suggested that ion composition may be a factor in toxicity, not just total ionic salt content. Subsequent bioassays were performed with single salts and with mixtures representing major ion combinations present in the OSPW (carbonate, sulphate, chloride and sodium). The interaction between NAs and salinity was assessed by exposing both *C. dubia* and *D. pulex*, which is more tolerant to salt than

C. dubia, to mixtures of NAs extracted from OSPW and relevant major ions chosen based on results from the salt toxicity tests.

In-situ caging of wood frog (*Rana sylvatica*) larvae in wetlands formed from oil sands tailings materials (OSPM) (PO)

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Extracting oil from oil sands produces large quantities of waste tailings water and solids (sand and clay), which may be reclaimed through formation of wetlands. To understand the impact of these wetlands, a mesocosm study of indigenous amphibians (wood frogs) was carried out during the spring and summer of 2006 and 2007. In both years, frogs were exposed to wetlands containing oil sands process-affected materials (OSPM) and reference wetlands (no OSPM). In year 2 there were 14 sites; 7 reference, and 7 OSPM- affected sites, which were classified as old ($\geq$ 7 yrs) or young ($\leq$7 yrs). Four enclosures, with 50 tadpoles each, were placed in each wetland. Survival ($p=0.004$), time to metamorphosis ($p\leq 0.001$), and thyroid hormone ratios ($p=0.005$) of larvae raised in young OSPM wetlands were different from those of reference wetlands and old OSPM wetlands. Other data to be presented include liver EROD activity and whole-body triglyceride concentrations. These results suggest that the formation of wetlands from tailings material may support populations of amphibians if the wetlands are allowed to age long enough. The development and health of native amphibians reflects the sustainability of the different reclamation strategies and details the impacts of OSPM to higher trophic level organisms.

GENERAL AQUATIC TOXICOLOGY

Aquatic toxicology of perfluorooctane sulfonate and related fluorochemicals (PL)

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Perfluorinated compounds are released into the environment via their use in wetting agents, lubricants, stain resistant treatments, and foam fire extinguishers. Perfluorooctane sulfonate (PFOS) is the terminal breakdown product of many commercially used perfluorinated compounds. PFOS is resistant to chemical and biological changes and does not significantly degrade under environmental conditions. PFOS has little mobility in the environment due to low volatility and strong soil adsorption. In laboratory and field tests, PFOS has been shown to bioconcentrate in fishes. Toxicity studies involving plants, invertebrates, and vertebrates from both terrestrial and aquatic habitats have been conducted with PFOS. Based on these toxicity studies, concentrations of PFOS were calculated for surface waters that are protective of aquatic plants and organisms. In addition, a critical body residue of PFOS was calculated for fish. Additional toxicity testing is needed to develop safe PFOS concentrations for terrestrial wildlife.

Toxicity of nano-sized titanium dioxide (TiO_2) on the freshwater green algae species *Pseudokirchneriella subcapitata* (PL)

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Nano-sized titanium dioxide (nano-TiO_2) as well as other nano- metal based oxides has been intensively used for antimicrobial purposes, and in soil and water remediation processes because of their absorbent or photocatalytic properties. The continued use of nano-TiO_2 in sunscreen lotions and other nano-based personal care products may lead to an increased environmental burden, and ecotoxicological effects are not well known. Some studies have recently demonstrated that exposure to nano-TiO_2 may have adverse effects on some aquatic receptors such as daphnia and algae. Although the exact mechanism of toxicity of nano-TiO_2 is not known, we tested the hypothesis that the toxicity of nano-TiO_2 was related to the size and surface area of the nanoparticle. We evaluated the toxicity of nano-TiO_2 having different sizes and types, using the *Pseudokirchneriella subcapitata* 96 h-algae growth inhibition test. Results indicated that nano-TiO_2 toxicity was related to the concentration, size and surface area of the nanoparticle. This study present toxicity of different sizes and types of nano-TiO_2, and investigate the potential role of ROS generation.

Investigating the sensitivity of freshwater mussel larvae to chloride salts: evaluating the potential threat to endangered species (PL)

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Glochidia, the larvae of freshwater mussels (Unionidae, Bivalvia), were found to be sensitive to chloride salts. The sensitivities of glochidia to sodium chloride and potassium chloride were assessed in reconstituted moderately-hard water (170 mg $CaCO_3 \cdot L^{-1}$) using an acute (24 h) standardized (ASTM) toxicity test. The resulting EC50s (with 95 % Confidence Intervals) for NaCl were 277 (243-311) $mg \cdot L^{-1}$ for *Lampsilis siliquoidea* (Fat Mucket) and 166 (80-249) $mg \cdot L^{-1}$ for the endangered species, *Lampsilis fasciola* (Wavy-rayed Lampmussel) (EC50s based on nominal concentrations). Glochidia were also extremely sensitive to KCl, with EC50s of 20 (18-22) $mg \cdot L^{-1}$ for *L. siliquoidea*, and 18 (16-20) $mg \cdot L^{-1}$ for *L. fasciola*. Given these results, there is a potential that increased salination of surface waters may have or will pose a threat to the successful reproduction of freshwater mussels, including the endangered species. Data from the Grand River Conservation Authority report chloride levels between 13 and 103 $mg \cdot L^{-1}$ in this important mussel habitat between May and September when many species of mussels release glochidia. Also, the Provincial Water Quality Monitoring Network of Ontario report potassium levels between 1 and 9 $mg \cdot L^{-1}$ in this watershed. It is reasonable to suggest that current levels of chloride and potassium may pose a threat to glochidia survival in Southern Ontario Rivers. Field studies are ongoing to assess glochidia survival in field-collected waters (Grand River Watershed) in relation to measured levels of potassium and chloride.

Effects of acute and chronic salt exposure on early life stages of amphibians (PL)

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The effect of roads on populations and communities is a major issue in amphibian conservation. Habitat loss and isolation, direct mortality, chronic disturbance, and toxic chemicals in runoff water are known to negatively affect amphibians inhabiting roadside wetlands. Road salt in runoff water has resulted in elevated chloride concentrations in many freshwater systems. Excessive chloride is known to damage ecosystem structure and function. The purpose of this research was to investigate the effects of acute and chronic NaCl exposure on early developmental stages of amphibians. Toxicity tests were performed on eggs and larvae of multiple amphibian species native to Nova Scotia to assess effects on growth, development, survivorship, and behaviour. Acute salt exposures indicated that spotted salamanders and wood frogs were the most sensitive to elevated chloride concentrations. Chronic exposures at environmentally significant salt concentrations resulted in reduced hatching success, extended larval period, increased mortality, and developmental and behavioural anomalies in most amphibian species. A differential response to elevated chloride concentrations among amphibian species potentially structures amphibian communities in roadside wetlands.

Diagnosing and analyzing triangular relationships in environmental toxicology (PL)

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In triangular relationships, *Y* values vary over a wide range at one end of the *X* scale, but variance of Y decreases and *Y* values approach a minimum or maximum at the other end of the *X* scale. Distributions of *Y-X* points resemble a right triangle. Triangular relationships are common in environmental toxicology, particularly in field studies. Typically, a wide range of responses (*Y*) in laboratory toxicity tests or field surveys from "Good" to "Bad" occurs at low *X* values (e.g., low contaminant concentrations or exposure levels), but a narrower range of mostly "Bad" responses occurs at higher *X* values. Variances of predicted *Y* values from parametric regressions increase or decrease as *X* increases (=heterogeneity of variance, a violation of one assumption of parametric regressions). Parametric regressions also predict mean or median responses, which may not be of interest. Possible causes of triangular relationships are considered, and examples and methods for diagnosing and analyzing triangular relationships are provided. Triangular relationships can best be diagnosed and analyzed by regressing percentiles of *Y* on *X*. The simplest approaches can be conducted in Excel; more complex methods require specialized statistical programs. The "best" approach depends on the project purpose and the robustness of the available data. Simple methods are adequate to diagnose and describe triangular relationships, especially for large data sets. Complex methods are often required for quantitative purposes (e.g., hypothesis testing, risk assessment, modelling) and/or smaller data sets.

Identifying the cause(s) of toxicity at sites in a stormwater management facility to the freshwater amphipod *Hyalella azteca (PL)*

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The Terraview-Willowfield stormwater management facility (Toronto, Ontario) receives inputs of multiple contaminants, including road salt, PAHs, and metals, via run-off from

Highway 401 (a multi-lane, divided highway with controlled access) and the surrounding residential area. The Sediment Quality Triad was used in a previous study to assess the ecological condition of the two stormwater ponds in this facility. It was determined that while PAHs and metals in the sediments contributed to poor habitat quality, salinity in the overlying water appeared to further influence the toxicity of certain sites in deeper sections of the ponds. In order to investigate the effects of salinity in these ponds to benthic invertebrates, overlying water and sediment samples were collected from 4 sites in the fall of 2007 (low salinity conditions) and 6 sites in the spring of 2008 (high salinity conditions). Four-week sediment toxicity tests were conducted in Imhoff settling cones with the freshwater amphipod, *Hyalella azteca*. Survival and growth of *Hyalella* in the fall samples (200 mg $Cl^{-}\cdot L^{-1}$) were variable; no clear pattern of toxicity was evident from a preliminary examination of the results. However, effects on survival and growth of *Hyalella* in the spring samples were observed at 1400-4000 mg $Cl^{-}\cdot L^{-1}$. These effects will be compared to chloride and PAH levels in the overlying water, as well as bioaccumulation of metals, in order to determine the cause(s) of toxicity.

The costs and management of hypoxia in salmon farming (PL)

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Newfoundland Salmon aquaculture industry is vastly expanding to new sites on the island south coast with a promising economic boost to the rural communities affected by diminished fisheries. The environmental characteristics of those sites are vital to this development. The physical characteristics of the water especially temperature, dissolved oxygen and salinity have a limiting effect on the site viability and productivity. The physiological effects of hypoxia in fish have been well documented. Around most farm sites oxygen levels vary diurnally and seasonally. During summer, dissolved oxygen levels are at their lowest due to higher water temperatures, higher feeding rates, and increasing biomass. All of these factors make the period from July to September critical for oxygen depletion. Hypoxia may have adverse effects on weight gain, feed consumption, feed conversion, reproductive performance in broodstock animals, disease resistance, efficacy of orally-administered therapeutants, and mortality after episodes of infectious diseases. These effects can adversely impact the commercial viability of farms, production and harvest scheduling, and the choice of husbandry measures to minimize these effects. This presentation describes the costs of hypoxia and its economical impact, as well as the strategies that may be employed to mitigate and manage this problem.

Predictive modelling provides answers to public scrutiny of marina development on the Shuswap Lake system (PL)

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Land development pressure is proceeding at an unprecedented rate along lakeshores of southern British Columbia (BC). The general public is concerned and educated about environmental impacts from land development activities. Public scrutiny is intense when it comes to development on the Shuswap Lake system, including Mara Lake. Environmental impact assessments rely on predictions of potential impacts based on perceived environmental sensitivities and anticipated changes to the natural environment. Predicting impacts has traditionally used mainly interpretations by professionals of potential changes to the physical environment. However, biological opinions are not enough to answer questions about water quality and fish habitat effects with sufficient level of assurance. An example will be presented where several modelling techniques provided predictions of potential effects on the physical processes within a region of Mara Lake, BC. Untreated grey water from large boats, alteration of water circulation and sediment movement by marina placement, and boat launch construction were modeled using proprietary three dimensional time-stepping computer models. The predictive models provided enough evidence to convince developers to alter marina and boat launch configurations to avoid or minimize negative environmental effects. This allowed biologists to complete an impact assessment with a higher level of confidence so that impacts were avoided or minimized, resulting in a sustainable marina development with positive public benefits.

Parasites and their potential effects on aquatic hosts in ecotoxicological studies (PL)

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Parasites are integral part of aquatic ecosystems. They can be found in almost any water body and at any trophic level. Their life-cycles involve one or more host organisms, and many hosts harbour a number of different parasite species and individuals. Although it is well known that parasites affect life-history traits (e.g., survival, reproductive fitness) of their hosts, their occurrence and potential impact is often neglected in ecotoxicological studies. This presentation gives an overview on some parasites of aquatic organisms and their modifying effects on physiology and life-history of their hosts in the presence of environmental pollutants.

Re-assessment of liver tumor incidences in wild fish from Canadian Areas of Concern (PL)

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In 1987, the International Joint Commission identified 43 Areas of Concern (AOC) in the US-Canadian Great Lakes. To qualify as an AOC, the area contained one or more beneficial use impairment (BUI). One of the BUI's that was identified at a number of locations was fish tumors or other deformities. It was defined as when the incidence rates of fish tumors or other deformities exceed rates at unimpacted control sites or when survey data confirm the presence of neoplastic or preneoplastic liver tumors in bullheads or suckers. Over the last several years Environment Canada has undertaken studies in Canadian AOCs to determine the current state of fish and wildlife health. Collaborations were developed with specialists at the Department of Fisheries and Oceans who were experts on histological techniques in order to evaluate properly data collected on tumour presence. Phase One was focusing on conditions in AOCs of the lower Great Lakes. This presentation will compare liver tumour incidences with those of specific reference sites sampled for the various AOC studied. It will also evaluate the use of lake wide reference site data in order to assess the relevance of the findings at individual AOCs. This data will be used by individual AOCs in order to determine whether improvements have occurred within the AOC and whether it can eventually be delisted.

Does feeding ecology influence hydrocarbon patterns in British Columbia sea otters (*Enhydra lutris*)? (PL)

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Oil pollution has been identified under the Species at Risk Act (SARA) as the greatest threat faced by British Columbia (BC)'s 3 200 sea otters (*Enhydra lutris*). We measured alkane and polycyclic aromatic hydrocarbon (PAH) concentrations in blood samples taken from 29 live-captured sea otters (grouped as males, females, and juveniles) from central BC (Bella Bella) and the west coast of Vancouver Island (Nootka Sound). Alkane and PAH concentrations were highest in adult males from Bella Bella, possibly reflecting local hydrocarbon sources or differences in feeding ecology. Alkane patterns at both sites were characterized by an inverted bell shape, peaking at chain length C26 - C27. PAH patterns varied, but were dominated at both sites by naphthalene and its methylated derivatives (C1-C4), and methylated fluorenes (C1-C3). In Nootka Sound, alkane (C22 – C31) concentrations were significantly related to estimated age (males: r = -0.88; p = 0.004; females: r = 0.70; p = 0.03), although contrasting trends between males and females may reflect sex-based differences in metabolism and/or feeding ecology. However, alkane concentrations did not change with age in Bella Bella males, suggesting that prey availability or selection may be a dominant factor in alkane accumulation in sea otters. PAHs did not vary with age or $\delta^{15}N$ at either site, suggesting that factors other than age or feeding ecology affect their pattern in sea otters. This first report of hydrocarbons in BC sea otters provides an important baseline signature of current natural and anthropogenic sources in the face of a burgeoning shipping sector and in advance of possible offshore oil and gas exploration and development.

Decline in antioxidants such as Vitamin E and reduced glutathione over time as rainbow trout (*Oncorhynchus mykiss*) embryo develops (PL)

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We are assessing the effect of CEWAF (Chemically Enhanced Water Accommodated Fraction) of Federated crude oil on the oxidative defence capacity of rainbow trout (*Oncorhynchus mykiss*) in their early life stages (Day 0- Day 60 post-fertilization; i.e., fertilization until the onset of feeding). CEWAF of Federated crude, mimics the solubilization of polynuclear aromatic hydrocarbons (PAH), the components of oil that are embryotoxic, by high-energy mixing during an oil spill. Indications of oxidative defence capacity were measured as the concentrations of Vitamin E and reduced glutathione in tissues of embryonic trout exposed to the CEWAF. Treatments of retene (model embryotoxic PAH; positive control), water (negative control), and dispersant only, were also used to demonstrate that any effects on the oxidative defence capacity of embryos were due to oil exposure. As the embryos develop, they will use up finite stores of antioxidants such as Vitamin E. Exposure to CEWAF and retene should increase the rate of depletion of anti-oxidants and may induce a greater oxidative stress. Hence, with increased exposure time, Vitamin E and reduced glutathione level in both CEWAF- and retene-exposed trout embryos should show the greatest decline. After the onset of feeding, the oxidative stress capacity should be enhanced as indicated by Vitamin E concentrations and the percentage of glutathione in its reduced form.

Up-regulation of hepatic Abcc2, Abcg2, CYP1A1 and GST in mummichogs *(Fundulus heteroclitus)* from the Sydney Tar Ponds, Nova Scotia, Canada (PL)

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Aquatic organisms prevent bioaccumulation of pollutants by cellular detoxification mediated by phase I and II enzymes and ATP-binding cassette (ABC) transporters. The relationship among ABC transporters Abcb1/Mdr1, Abcb11/Spgp, Abcc2/Mrp2, Abcg2/Bcrp, phase I enzyme CYP1A1 and phase II enzyme GST-mu was investigated by quantifying hepatic transcript abundance in mummichogs (*Fundulus heteroclitus*) from the Sydney Tar Ponds, Nova Scotia, Canada, a site heavily polluted with polycyclic aromatic hydrocarbons, polychlorinated biphenyls and heavy metals. In Tar Pond mummichogs, hepatic mRNA levels of Abcc2, Abcg2, CYP1A1 and GST-mu were elevated compared to reference sites, suggesting that hydrophobic contaminants undergo phase I and II metabolism and are then excreted into the bile of these fish. Hepatic Abcb1 and Abcb11 mRNA were not up-regulated in fish from the Tar Ponds compared to two reference sites. The results suggest that up-regulation of phase I and II enzymes and complementary ABC transporters may confer contaminant resistance to Tar Pond fish.

Time course of the expression of biomarkers during the manifestation of lethal effects in *Elliptio complanata* mussels exposed to aeration lagoons treating domestic wastewaters (PL)

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The time course in the expression of sublethal effects that precede mortality events in mussels exposed to municipal wastewaters is not well understood. Our study thus sought to examine a time course in biomarker responses of freshwater mussels exposed to two final aeration lagoons for the treatment of domestic wastewater. Mussels were caged in two terminal aeration ponds (AL1 and AL2) for different time periods (1, 15, 30, 40 and 60 days) and examined for biomarkers representative of biotransformation, metabolism of endogenous compounds, tissue damage and gametogenesis. Results showed that mortality events occurred after day 29 of exposure, gradually reaching 30 % and 45 % mortality for AL1 and AL2, respectively at day 62. These aeration lagoons were estrogenic, as evidenced by the rapid induction of vitellogenin-like proteins, and they were also good inducers of cytochrome P4503A activity, considered as one of the major drug metabolizing enzymes. Several biomarkers were expressed before the manifestation of mortality (vitellogenin-like proteins, aspartate transcarbamoylase, metallothioneins, monoamine oxidase, hemoprotein oxidase, cytochrome P4503A activity, glutathione S-transferase, DNA strand breaks and mitochondrial electron transport activity), while others were expressed during mortality events (xanthine oxidoreductase, cytochrome P4501A1 activity, lipid peroxidation, gonad lipids and cyclooxygenase). A factorial analysis revealed that temperature-dependent mitochondrial electron transport activity, hemoprotein oxidase, monoamine, and aspartate transcarbamoylase had the highest factorial weights. Furthermore, canonical analysis of biomarkers revealed that reproduction (gametogenesis) was more significantly related with those linked to tissue damage and biotransformation, while energy status (i.e. increased energy expenditure and decreased lipid reserves) was not significantly related to gametogenesis or tissue damage in AL1, the least toxic and estrogenic lagoon. In AL2, the more toxic and estrogenic lagoon, energy status was significantly correlated with tissue damage and gametogenesis, in addition to biotransformation activity and metabolism of endogenous substrates. Domestic wastewaters treated in aeration lagoons display a complex pattern of sublethal responses in mussels prior to the manifestation, in some instances, of mortality events.

Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near corn fields: evidence of exposure by bacterial ingestion (PO)

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The purpose of this study was to examine the contamination of *cry1* and *cry1Ab* genes from *Bacillus thuringiensis* and transgenic corn in feral freshwater mussels collected from sites located in proximity of corn fields. In addition, mussels were transplanted for 2 months to a site in the Huron River, upstream to the Richelieu River, which is subject to intensive corn farming. Mussels were significantly contaminated by both genes in their gills, digestive glands, and gonads, as determined by qPCR methodology. Gene sequence analysis confirmed the presence of transgenic corn *cry1Ab* gene in mussel tissues. In an attempt to explain the presence of the transgene in mussel tissues, heterotrophic bacteria were grown from surface water and sediment samples on agar plates in the Richelieu River in May and August. The transgene was found at two out of six surface water samples and in one sediment sample. A significant correlation was obtained between the levels of *cry1* from Bt and *cry1Ab* from transgenic corn. The study revealed that exposure to transgenic corn *cry1Ab* gene in mussels seems to proceed by ingestion of microorganisms during feeding.

Effect of produced water on cod (*Gadus morhua*) sperm cells and fertilization (PO)

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Produced water, a by-product of offshore oil production, contains significant amounts of alkylphenols that can cause endocrine disruption. Reproductive dysfunction resulting from exposure to low concentrations of pollutants can be caused either by direct action on the gametes, or indirectly by modulation of the endocrine system. Atlantic cod *(Gadus morhua)* are pelagic spawners with external fertilization and no parental care. Because fertilization occurs in the water and cod spermatozoa can stay motile and fertile for a long time, sperm characteristics are of the most importance for determining fertilization success. PW dispersion modeling shows that dilution by at least 240 times occurs within 50-100 m, and up to 9000 times by 20 km from the discharge. We have investigated the *in vitro* effect of PW on sperm cells by exposing spermatozoa to 100 $mg \cdot L^{-1}$ (x10, 000 dilution), 200 $mg \cdot L^{-1}$ (x5000) or 500 $mg \cdot L^{-1}$ (x2000) of PW. We have assessed fertilization rates, viability, respiration, ATP levels, total motility times and enzymatic responses (citrate synthase, catalase, lactate dehydrogenase, and lipase) of sperm cells exposed for 1 hour to these realistic concentrations of PW. Other than a non-significant decrease in enzymatic activities and fertilization rates, our results show no strong effect of diluted PW on any of the parameters investigated. This study highlights the importance of further exploring effects of PW on reproduction by focusing on the endocrine system and suggests that PW components have no direct effect on sperm cells.

The acute toxicity of fluorotelomer acids to several North American freshwater organisms (PO)

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Perfluorinated acids (PFAs) are used globally in a wide variety of industrial and commercial surface treatment applications, and their global recalcitrance and pervasiveness have made them the focus of recent scientific and public concern. Current evidence suggests that many PFAs form in the environment through a degradation pathway beginning with the fluorotelomer alcohols, with the fluorotelomer carboxylic acids (FTCAs) as an important intermediate degradation product. Preliminary research has shown that FTCAs can be two to three orders of magnitude more toxic to freshwater organisms than their PFA counterparts; however, additional data on a broader spectrum of organisms is required in order to fully evaluate their relative risk to the environment. We investigated the toxicity of 6:2, 8:2 and 10:2 saturated and unsaturated FTCAs to two species of freshwater algae, *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*, and the amphipod *Hyalella azteca*. Preliminary tests have indicated a reproductive EC50 >50 $mg \cdot L^{-1}$ for the two algal species, and an LC50 *H. azteca* for the 8:2 FTCA. Results for the tests using both saturated and unsaturated acids on these species will be presented, and future work will focus on understanding the mechanism of action of the FTCAs and their role in reproductive inhibition and early life-stage development in vertebrate models.

Effects of acute lead exposure on essential ion uptake and homeostasis in *Daphnia magna* (PO)

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Lead contamination in natural waters is an issue of current regulatory concern throughout the world, including Canada. Waterborne lead is known to cause toxicity by disrupting ionic (e.g. Ca2+, Na+ and Cl-) homeostasis in freshwater fish. However, the understanding of physiological mechanisms of lead uptake and toxicity in aquatic invertebrates is lacking. Daphnids are extensively used for standard toxicological assays as a representative aquatic invertebrate since they are highly sensitive to many different contaminants, including metals. The main objective of the present study was to investigate the effects of acute lead exposure on essential ion uptake processes in *Daphnia magna*. The results indicated that waterborne lead inhibited waterborne calcium uptake in a concentration and time-dependent manner, and this interaction was predominantly competitive in nature. The pharmacological assessments indicated that waterborne lead uptake was inhibited by both voltage-sensitive and voltage–insensitive epithelial calcium channel blockers. Moreover, waterborne calcium, but not sodium or magnesium, reduced waterborne lead accumulation in *D. magna*. In addition to calcium, waterborne lead also inhibited waterborne sodium uptake in a time-dependent manner. Overall, the results suggested that lead uptake occurs via both voltage-sensitive and voltage-insensitive epithelial calcium channels, and acute waterborne lead exposure causes disruption of calcium and sodium homeostasis in *D. magna*.

The toxicity of oil-contaminated muskeg following biodegradation (PO)

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Contaminated soil from crude oil pipeline spills must be remediated in accordance with environmental regulations. The current Canadian Council of Ministers of the Environment criteria indicate the maximum allowable levels of hydrocarbons resulting from an oil spill. However, the criteria assume that all detectable hydrocarbons are petroleum hydrocarbons (PHC) and do not account for naturally-occurring biogenic hydrocarbons (BHC). Because of this failure to account for the naturally occurring BHCs, soils in question may be wrongfully assessed as being PHC contaminated. False identification of contaminated soils could lead to unnecessary bioremediation, which is costly and potentially disruptive to functioning ecosystems. This study is part of a larger project to distinguish between natural and petroleum F3 hydrocarbons in oil-spill-impacted muskeg material. The objective of the current study was to examine the toxicity of oil-contaminated muskeg (peat) following biodegradation in laboratory microcosms. Preliminary acute toxicity tests using locally purchased *Sphagnum* peat moss contaminated with Federated Crude oil (10,000 µg·g^{-1} Fraction 3; 22,000 µg·g^{-1} total petroleum hydrocarbons) had no effect on earthworm (*Eisenia andrei*) survival, whereas springtails (*Orthonychiurus folsomi*) were more sensitive. Crude-oil-contaminated peat (commercial) was tested using earthworm (*E. andrei*) and springtail (*O. folsomi*) reproduction bioassays and a Northern wheatgrass (*Elymus lanceolatus*) growth bioassay. The three test species will also be used to test for reduced toxicity following biodegradation of Federated Crude oil-contaminated muskeg (from northern Alberta) under simulated conditions.

Probabilistic neural networks modeling of the 72-hr EC50 acute toxicity endpoint to *Pseudokirchneriella subcapitata* (PO)

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Two large-scope models targeting prediction of the *Pseudokirchneriella subcapitata* 72-hr EC50 acute toxicity endpoint for organic compounds are presented. Both models are based on the Maximum Likelihood Estimation paradigm. The first is a basic PNN model with Gaussian Kernel and statistical correction included. The second is a PNN model with separated variables with Gaussian Kernel and statistical correction included. Both models are trained on the same data set consisting of 528 of measured and estimated *Pseudokirchneriella subcapitata* 72-hr EC50 acute toxicity end points. An external test set consisting of information on 46 additional compounds is used to evaluate the predictive performance of both models. The model descriptors are basic counts of atoms and specific fragments and the molecular weight. The basic PNN model with Gaussian Kernel and statistical correction included performed the best.

Effect of 3,3',4,4'-tetrachlorobiphenyl on thyroid hormones, sexual maturation, and EROD activity in male Atlantic cod undergoing winter fasting (PO)

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Atlantic cod accumulate high concentrations of persistent organic pollutants (POPs) in their lipid-rich liver and also fast during the winter months, when their condition declines. This marked seasonal variation in nutritional status could increase the risk of deleterious effects associated with these compounds. In this study, male cod (mean body length, 62 cm) were exposed to a single oral dose of 5 mg 3,3',4,4'-tetrachlorobiphenyl (PCB77) kg^{-1} body weight and then held for 16 weeks without food at a water temperature of 6 °C. Despite the lack of feeding, most cod were in relatively good condition (> 40 % lipids wet weight in liver) at the end of the fasting period. Concentrations of PCB77 in hepatic lipids did not change significantly as a function of lipid content. However, for highly chlorinated PCBs initially present in fish such as 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), concentrations increased as lipid content decreased. Hepatic EROD activity, hepatosomatic and gonadosomatic indices and plasma thyroid hormones increased with PCB77 exposure, though at concentrations several orders of magnitude higher than those found in St. Lawrence Estuary's cod. Levels of 11-ketotesterone in plasma and hepatic lipid content were not affected by PCB77 exposure. In conclusion, large male Atlantic cod from the St. Lawrence Estuary appear fairly resistant to PCB77 toxicity, even at the end of a 16-week fasting period. However, loss of condition during experimental fasting was moderate. Thus, the effect on POPs toxicity of a more severe loss in condition, similar to that experienced in the northern Atlantic cod stocks in the wild, should be further studied.

Investigating the reasons for sediment toxicity in the St. Marys River (PO)

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A study performed by Environment Canada in 2002 in the St. Marys River identified sediments as being toxic to the midge, *Chironomus* and the mayfly, *Hexagenia* in standard laboratory bioassays. Toxicity (growth effects) to *Hexagenia* appeared to be partially related to sediment petroleum hydrocarbon concentrations; however, it was not clear what was causing acute toxicity to *Chironomus*. This study further investigated reason(s) for sediment toxicity by quantifying bioavailability of contaminants of concern (e.g., polycyclic aromatic hydrocarbons (PAHs), metals) in laboratory-exposed organisms and toxicity identification evaluation (TIE) methods were applied to the sediments by the USEPA. The TIE used 10-d solid phase toxicity tests with freshwater organisms, the amphipod, *Hyalella* and *Chironomus*. The relative effectiveness of each TIE amended sample and subsequent toxicity test provides information on the type(s) of chemicals responsible for toxicity. Laboratory bioassays were performed at 12 sites which provided further spatial coverage in delineating toxicity in the affected area. Toxicological response and tissue contaminant concentrations were compared to local and regional reference sites. Toxicity was examined in both sieved and un-sieved sediments to determine if sediment manipulation (pre-sieving) could have affected 2002 test outcomes. Toxicity-contaminant relationships were explored by regression analysis. Overall toxicity was more severe in tests with un-sieved sediments (including co-tested reference sediment); therefore, pre-sieving may underestimate toxicity: 11 % of sites were toxic with sieved sediment while 33 % were toxic with un-sieved sediment. PAH measurements in mayflies demonstrated increased bioavailability at test sites (by an order of magnitude), compared to reference sites; however, biological effects did not appear related to sediment or tissue PAH concentrations (as total PAHs or individual compounds). The TIE study may shed further light on cause(s) of toxicity.

Mechanisms of relaxation in dorsal and ventral aorta in normal and benzo[a]pyrene-exposed trout (PO)

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Compared to mammals, vasorelaxation in fish is poorly characterized. We hypothesized that prostaglandin E_2 (PGE_2), sodium nitroprusside (SNP), and sodium hydrogen sulfide (NaSH) would mediate vasorelaxation in fish and that toxicant-exposure would alter their effects. Rainbow trout (*Oncorhynchus mykiss*) dorsal and ventral aorta ring responses to vasoactive agents were characterized in an organ bath. In untreated fish, carbachol contracted ventral aorta and calcium ionophore (A23187) contracted dorsal aorta, while SNP and PGE_2 relaxed both vessels. NaSH contracted ventral aorta, but relaxed dorsal aorta. After benzo[a]pyrene (BaP) injections, PGE_2 responses in dorsal aorta were unchanged. However, SNP caused a significantly larger relaxation in dorsal aorta from BaP compared to vehicle-injected fish, while NaSH-induced relaxation was eliminated in BaP-exposed fish. BaP exposure increased hepatic, but not aortic, CYP1A enzyme activity in BaP compared to vehicle-exposed fish. In conclusion, exposure to the toxicant, BaP, appears to alter vasorelaxation in trout dorsal aorta.

Acute exposure to 2,4-dinitrophenol alters swim performance in zebrafish (PO)

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Examination of fish swim performance is a potentially environmentally relevant, non-lethal endpoint for examining fish survivability. Zebrafish served as the model to investigate the acute affect on swim performance of a toxicant that acts as an oxidative uncoupler. Fish (n=10 per group) were aqueously exposed in a static medium to 0, 6, and 12 $mg \cdot L^{-1}$ 2,4-dinitrophenol for 24 hours. Critical swimming speed (U_{crit}, a measure of swimming speed and endurance) was determined using a Loligo Systems mini swim tunnel in clean water. Fish were recorded at various intervals of U_{crit} with a high speed camera. The acquired images were used for swim motion analysis. No fish died during the control exposure. Mortalities in the 6 and 12 $mg \cdot L^{-1}$ exposure groups were 20 and 30 percent, respectively. Ucrit was significantly decreased

($p<0.05$ in Tukey's test after 1-way ANOVA) in fish exposed to 12 $mg \cdot L^{-1}$, but not 6 $mg \cdot L^{-1}$ dinitrophenol. However, the oxidative uncoupler did not have a significant effect on swim motion (e.g. maximum amplitude of tail bend, angle of tail bend and length compression during swim stroke). In conclusion, this study shows that acute exposure to a mitochondrial electron transport chain uncoupler can affect swim endurance (U_{crit}). Since swim performance is linked to both predator evasion and food acquisition, this may result in a decrease in fish survivability.

Does oral exposure to individual HBCD diastereoisomers alter thyroxine (T4) metabolism in juvenile rainbow trout? (PO)

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Hexabromocyclodecane (HBCD) is the principle fire retardant in polystyrene foams that are used as insulation in the building industry and for upholstering furniture. The mixture consists of three diastereoisomers, α, β and γ. While the γ isomer is most often detected in abiotic compartments, there are differences in the composition of HBCD residues that are measured in biota. There is also little information on the relative potency of the diastereoisomers to induce biological effects in exposed organisms. Our earlier work suggested that fish exposed to HBCD exhibited an increase in the rate of turnover of thyroxine (T4), the precursor molecule to the active thyroid hormone triiodothyronine (T3)[1]. To examine this further, juvenile rainbow trout (*Oncorhynchus mykiss*) were held in the laboratory and fed diets containing environmentally relevant concentrations of the individual α, β and γ isomers. Thirty two days after feeding began, 20 fish from each group were individually anesthetized, weighed, measured and then dosed by oral gavage with gelatin containing [^{125}I] T4. Measurements of the labeled T4 two days after gavage provided a means of determining tissue disposition and elimination rates of the hormone. By determining concentrations of native hormone in plasma and liver, ratios relative to the labeled dose in those tissues also provided a measure of turnover rates. Two days post gavage the β-HBCD diastereoisomer increased the deposition of [^{125}I] T4 in bile while pre-exposure to the γ-isomer reduced retention of [^{125}I] T4 in intestine and viscera. Monitoring of [^{125}I] T4 in tissues will continue at 4, 6 and 8 days post gavage to examine the potential for individual HBCD diastereoisomers to increase turnover rates of thyroid hormones and disrupt the thyroid axis.

The effect of lowered dissolved oxygen on fathead minnow reproduction (PO)

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Dissolved oxygen (DO) is integral to most aquatic organisms. While diel fluctuations in DO concentration are a normal occurrence in aquatic ecosystems, anthropogenically-produced periods of prolonged hypoxia have the potential to cause reproductive impairment, reduced growth, and hormonal changes in fishes. In the current study, fathead minnow (*Pimephales promelas*) reproduction was quantified following exposure to several concentrations of dissolved oxygen. Using a custom-built system that is able to maintain DO concentrations at precise levels, reproductive performance was analyzed under 3.5 $mg{\cdot}L^{-1}$, 4.5 $mg{\cdot}L^{-1}$, 5.5 $mg{\cdot}L^{-1}$ and a control of 7.5 $mg{\cdot}L^{-1}$ DO. The results indicate that breeding attempts cease altogether at 4.5 $mg{\cdot}L^{-1}$ and lower. At 5.5 $mg{\cdot}L^{-1}$, breeding attempts and egg production occurred, but were significantly reduced from the control. Occurrence of courtship behaviour in the lower two treatments was also statistically lower than in the control. No significant differences, however, were observed from histological analysis of gonad tissue, and levels of testosterone, estradiol, and vitellogenin showed no significant difference between treatments. The results of this study demonstrate that reproductive behaviour may represent a more sensitive early marker of reproductive impairment than hormonal or histological analysis. These results additionally have potential for application in the restoration of aquatic systems with artificially lowered DO.

Evaluation of hypoxia occurrence in salmon aquaculture sites in Fortune Bay, Newfoundland (PO)

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Three Atlantic salmon marine cage sites at Fortune Bay, Newfoundland have been monitored by Fisheries and Oceans Canada in collaboration with fish farmers and the Provincial Department of Fisheries and Aquaculture during the summer-fall of 2006-2007 using real-time water quality monitoring equipment. The study objectives were to evaluate the water physical characteristics and the variability of dissolved oxygen (DO) concentration over time, the relationship with other water quality variables and the identification and characterization of hypoxic events on the monitored sites. The study reported that while one site suffered from an extended period of hypoxia (i.e. DO < 6 $mg{\cdot}L^{-1}$), intermittent hypoxic events with a mean duration of approximately 2 hours reported at the other two monitored sites when fish are present on the site. No such events were recorded on one of the same sites when it left to fallow. Exposure to intermittent hypoxia could result in physiological consequences not always well

known in fish. Given observed deterioration of linear relationships between dissolved oxygen concentration and other water quality parameters (e.g. temperature, salinity) at hypoxic conditions, the dedicated collection of continuous water quality data would support the in-depth examination of complex mechanisms controlling hypoxia and will aid fish farmers to develop real-time mitigating measures in such circumstances.

Determination of V_{max} and K_m for validation of an *in vitro* trout S9 fraction assay to predict *in vivo* fish metabolism of chemicals (PO)

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Bioaccumulation assessment for chemical substances is increasingly required by government regulators. Of 11,500 chemicals examined from Environment Canada's Domestic Substances List, DSL, only 352 have bioaccumulation data. REACH legislation will require bioaccumulation evaluation of chemicals based on tonnage use. The bioconcentration factor (BCF) determines the potential of a substance to bioaccumulate typically in fish. To support incorporation of *in vitro* metabolism data into BCF assessments, a model has been developed to extrapolate *in vitro* metabolism to whole fish biotransformation rates (k_{MET}) to refine BCF computer model predictions based on log Kow alone (Cowan-Ellsberry et al., 2008). The *in vitro* method consists of the hepatic S9 fraction from the rainbow trout, *Oncorhynchus mykiss* incubated with substrate at environmentally relevant temperatures. This study was designed to determine enzyme kinetic parameters to aid in substrate concentration selection for validation of the *in vitro* method. This includes determination of the Michaelis-Menten V_{max} (maximum enzyme velocity) and K_m (concentration at half of V_{max}) for each of the proposed test materials; which include chlorpyrifos, 4-nonylphenol, starane F, pyrene, methoxychlor and dibenzylether. These parameters (V_{max} and K_m) were determined by generating data for substrate depletion over time at various concentrations of the substrate. The values of the K_m will be used to determine the concentration of the substrate, approximately ½ K_m, for the method validation tests. Funding is provided by European Commission JRC/IHCP/In Vitro Toxicology Unit - ECVAM, Contract #CCR.IHCP.C434207.X0 and by European Chemical Industry Council Long-range Research Initiative (CEFIC-LRI), Contract #LRI-ECO6.2-ILSIHESI-0804.

Activated carbon - duct tape for treatment based toxicity reduction studies (PO)

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Our treatment based toxicity reduction approach seeks to employ multifunctional treatments such as activated carbon to deal with complex mixtures of variable and in some cases unknown toxicants. Activated carbon may contribute to toxicity reduction of complex effluents both through sorption of organic compounds and metals, and by providing o a reaction surface for oxidation and reduction reactions. This presentation will highlight specific applications of activated carbon in treatment based toxicity reduction studies for the power generation sector.

Effect of a freshwater oil spill on embryonic development in lake whitefish and northern pike (PO)

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Wabamun Lake (Canada) has been subject to ongoing contamination with PAHs from multiple sources for decades, and in August 2005 was exposed to ~149,500 L of bunker 'C' oil following a train derailment. We compared the pattern, frequency and severity of deformity in larvae of lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) incubated *in situ* in areas of Wabamun Lake exposed only to 'background' PAH contamination and in areas additionally exposed to PAHs from the oil. All sites in the lake showed incidences of deformity higher than are typically observed in laboratory studies. Lake whitefish at a small number of oil-exposed sites showed higher incidences of some deformities and a tendency to exhibit deformities of higher severity than sites not exposed to oil. However, we found few and relatively small differences in deformity of northern pike larvae among sites, and none that could be attributed to PAH exposure. Semipermeable membrane devices deployed with the eggs indicate that northern pike embryos (incubated in reed beds) were exposed to higher concentrations of dissolved PAHs than were lake whitefish (incubated on cobble), suggesting a difference between these species in uptake and/or sensitivity to PAHs.

Correlating gene expression with deformities caused by aryl hydrocarbon receptor agonists in zebrafish (*Danio rerio*) (PO)

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Aryl hydrocarbon receptor (AhR) agonists are known to cause lethal disturbances in fish development, but the precise mechanism has yet to be determined. We hypothesized that genes important for cardiovascular regulation (prostaglandin-endoperoxide synthases or PTGS) would exhibit a stronger link to deformities than detoxification enzymes (cytochrome P450 monooygenases or CYPs). Zebrafish (*Danio rerio)* eggs were exposed aqueously until 4 days post-fertilization (dpf) to the AhR agonists benzo(a)pyrene (BaP) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alone and in combination with the putative AhR antagonists resveratrol or alpha-naphthoflavone (ANF). Gene expression was measured using real-time, reverse transcriptase PCR in zebrafish at 5 and 10 (dpf). Mortalities did not differ significantly among groups at 10 dpf. However, deformities were significantly increased after BaP-ANF at 5 dpf and after BaP at 10 dpf, but not after TCDD treatment. Expression of several CYP and PTGS isozymes exhibited small, but statistically significant changes at 5 dpf, while expression was returned to control values by 10 dpf. Only CYP1A expression in TCDD-treated groups remained significantly higher at both time points. Neither resveratrol nor ANF antagonized TCDD-induced increases in CYP1A expression. Overall, CYP1A and PTGS-1 expression at 5 dpf were positively correlated with deformities ($r^2=0.44$ and $r^2=0.59$, respectively). All other genes examined were negatively correlated with deformities, particularly CYP1C2 ($r^2=0.68$). In conclusion, changes in CYP1A, CYP1C2, and PTGS-1 gene expression at 5 dpf are associated with developmental deformities, but further work is needed to determine which has the most important mechanistic link.

Alpine lakes as sentinels of change: tracking trends in accumulation of PFAs (PO)

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As alpine lakes are among the most extreme and remote ecosystems in the globe, they are particularly sensitive to environmental change. For this reason, they also function as model systems to track changes in contaminant accumulation. Perfluorinated acids (PFAs) are an emerging group of contaminants that have garnered much attention due to their toxicity, bioaccumulative potential and persistence in the environment. While these chemicals have been observed in remote locations of the globe, the pathways responsible for their long-range transport are still being debated. In addition, although production of PFAs by electrochemical fluorination was phased out in 2002, there is little information on how levels of PFAs in remote environments have changed over time. We have collected sediment cores from remote, high alpine lakes and reconstructed trends in PFA accumulation over the past century. The results will provide information on the presence and temporal changes in accumulation of PFAs, as well as the importance of atmospheric long-range transport in the distribution of these contaminants to remote alpine environments.

Nickel-induced alterations in some haematological and biochemical profiles of the Indian major carp (*Cirrhinus mrigala*) (PO)

M. Ramesh[1] *and M. Saravanan*[1]. [1]*Bharathiar University, Coimbatore, India*

INTRODUCTION

Metals from anthropogenic sources have been recognised as important contaminants in aquatic ecosystems. Physiological and biochemical biomarkers are frequently used for detecting or diagnosing lethal and sublethal effects in fish exposed to different toxic substances.

MATERIALS AND METHODS

The fish *Cirrhinus mrigala* was used in the present investigation. The physico chemical analyses of the water were estimated by the method of APHA (1998). The median lethal concentration (8.0 $mg \cdot L^{-1}$) of nickel sulphate for 24 h was calculated and 1/10th of the toxicant (0.8 $mg \cdot L^{-1}$) was taken for sublethal study. The haematological and biochemical parameters were analysed by using the following methods like Haemoglobin, Erythrocyte and Leucocyte Count, Plasma glucose and Plasma protein. The significance of treated fish was tested using student's 't' test. Statistical analysis was done by standard methods using software.

RESULTS

During sublethal concentration of the nickel sulphate for 15 days treatment period, erythrocytes count was decreased in experimental fish showing 54.44 % at the end of 5th and 68.36 % at the end of 15th day, respectively. The leucocyte count was increased in metal exposed fish showing 14.39, 58.55 and 119.15 % at the end of 5, 10 and 15th day, respectively. Haemoglobin level was decreased to 10.70, 19.61 and 32.83 % the end of 5th, 10th, and 15th day, respectively. The plasma glucose level was increased to 10.22 % at the end of 5th day and 54.01 % at the end of 15th day. The plasma protein level was decreased to 37.82, 49.56 % and 20.86 % at the end of 5th, 10th, and 15th day, respectively. Statistical analysis indicates that all the values were significant at 5 % level. In the present study, during acute exposure the fish showed various behavioural responses like erratic movements, wide-open operculum, a gradual retardation in their movement with extension of time, and profuse mucus production, etc. Finally, fish were apparently struggling hard for respiration and they died. The significant decrease of erythrocyte count might have resulted from a response of fish to ACTH increase as a result of stress caused by the presence of nickel. Further inhibition of erythropoiesis may be another reason for the decreased number of erythrocytes. The significant increase of WBC count indicates the protective mechanism of the fish against nickel toxicity or it may be due to increase in the population of neutrophils, acidophils and basophils. The observed low haemoglobin level can be related to the anemic condition of the fish, which may be due to disturbance of haemopoiesis through the presence of toxicant (nickel sulphate). The continuous elevation of plasma glucose level indicates the conversion of glycogen to blood glucose to supply energy to increased cell metabolism which is caused by the stress of nickel toxicity. The significant decrease of plasma protein level might have resulted from reduced protein synthesis or direct utilization of protein in gluconeogenesis to produce energy during stress. In the present investigation it is concluded that nickel sulphate at sublethal concentration has altered the haematological and biochemical parameters of the fish. The above-mentioned parameters can be effectively used as non-specific biomarkers against anthropogenic stress.

Predicting water-sediment interactions of uranium-spiked sediments with different overlying water chemistries to *Hyalella azteca* (PO)

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Studies with uranium (U) spiked sediments and five different overlying waters varying hardness and alkalinity independently, found that water pH, rather than calcium, predominately affects the dissolution of U from the sediment and U bioavailability and uptake in the amphipod *Hyalella azteca*. Calcium affected U accumulation through its effect on speciation rather than through direct competition with U for uptake. Experiments with caged animals suggest that U bioaccumulation and toxicity is mainly via the water rather than the sediment, while U bioaccumulation is a more reliable indicator of U toxicity than U concentrations in water or sediment. These water-sediment and water-bioaccumulation interactions were satisfactorily explained using saturation models. Currently we are trying to validate these models in field contaminated samples.

BARRIERS TO BIOLOGICAL RECOVERY IN METAL CONTAMINATED LAKES

Barriers to biological recovery in urban metal-contaminated lakes: Symposium introduction (PL)

J. Gunn[1], M. Butler[2], B. Keller[1], A. Merla[3], G. Morgan[1], C. Ramcharan[1], G. Watson[3] and N. Yan[4]. [1]Cooperative Freshwater Ecology Unit, Laurentian University, Sudbury, ON [2]Xstrata Nickel, Sudbury, ON [3]Vale Inco Ltd., Sudbury, ON [4]York University, Toronto, ON

The Sudbury case history has provided some of the best evidence in the world of the benefits of air pollution controls; it is now clear that significant water quality improvements have occurred throughout the greater Sudbury area as a result of extensive (>90 %) emission reductions. However, biological recovery has generally lagged far behind the chemical improvements due to the many confounding factors that affect recovery. This symposium focuses on the role of several of these barriers to recovery, including: residual metals, altered predator/prey communities, introductions of invasive species, loss of genetic stocks, disruption of terrestrial linkages (i.e. lack of organic matter inputs, excessive soil erosion) and the impacts of climate change. These objectives are addressed by linking lab and field experiments with extensive spatial and temporal (15-30 years) survey studies to produce foodweb models. Both standard and quite novel sampling and statistical methods are used to address the many interacting factors that affect recovery and to evaluate the benefits of potential management options such as land reclamation (soil amendments or tree planting) or piscivore introductions (fish stocking). The reference condition approach (RCA) is also applied, using both local and remote (Dorset, Ontario) reference lakes, to detect multiple stressor impacts in our experimental lakes.

Recovery of acidified, metal-contaminated lakes near Sudbury, Ontario, Canada (PL)

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Over 7000 lakes around Sudbury, Ontario, Canada were acidified by sulphur deposition associated with emissions from the Sudbury metal smelters and more distant sulphur sources. Many lakes close to the smelters were also highly metal-contaminated. Air pollution controls have led to widespread changes in damaged Sudbury lakes, including increased pH and decreased concentrations of SO4, metals and base cations. While chemical improvements have often been substantial, many lakes are still acidified, although water quality recovery is continuing. Biological recovery has been observed in some lakes, among various groups of organisms including fish, zooplankton, phytoplankton and benthos. Generally, however, biological recovery is still at an early stage. Studies of Sudbury lakes are providing insights into chemical and biological lake recovery processes when evidence of aquatic recovery from acidification is only starting to emerge from other acid-affected areas of the world. Lakes around Sudbury are also showing that the recovery of acid-damaged lakes is closely linked to the effects of other major environmental stressors such as climate change, base cation depletion and UV-B irradiance. Future studies of the recovery of acid-damaged lakes around Sudbury, and in other regions, will need to consider the interactions of these and other stressors.

Has liming fostered recovery of crustacean zooplankton in Sudbury's urban lakes? (PL)

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Sudbury's Middle, Hannah, Lohi and Clearwater lakes were acidified and metal-contaminated in the past. However, land and lake liming eliminated acidity in Middle and Hannah lakes, while reduced S emissions was followed by substantial water quality improvements in Lohi and Clearwater lakes. Crustacean zooplankton richness is a sensitive indicator of lake acidification. Here we use zooplankton richness to document biotic recovery in these four lakes, in particular comparing patterns of recovery in the limed lakes vs. the non-limed lakes, the latter with their more gradual water quality change and shorter circum-neutral time periods. Richness has increased in all the lakes, but the temporal dynamics and current state of recovery differed among lakes. The increase has been quite monotonic in Middle and Hannah lakes, less so in Lohi Lake, and typified by a large, early 1990s setback in Clearwater Lake. Richness has reached normal levels (8-12 species/standard count) in recent years in Middle and Hannah lakes, but has not yet consistently reached this target in Lohi and Clearwater lakes. The more complete recovery in Middle and Hannah lakes is surprising, because these lakes are the smaller of the four, they had higher initial and have higher current total metal levels, and lower initial zooplankton richness, all of which would be expected to reduce recovery rates. Reasons for these differences will be presented.

Effects of metal contamination and fish predation on the recovery of zooplankton in Sudbury Lakes (PL)

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In recent decades, lakes in the Sudbury area have shown significant improvements in water quality due to decreases in sulphur (S) and metal (Cu and Ni) emissions from area smelters and more distant sources. However, biological recovery seems to be lagging behind water quality improvements. The primary objective of this project is to examine the roles of two local factors, residual metal contamination and altered predation from fish communities, on zooplankton recovery in Sudbury area lakes. The secondary objective is to determine, if there is a generalized trajectory to zooplankton community recovery. Long-term records of zooplankton communities for some Sudbury lakes offer a unique opportunity to examine the relative effects of large changes in habitat quality (decreases in both acidity and metal concentrations) and dramatic changes in fish communities on crustacean zooplankton communities. We examine data collected over three decades on six study lakes; Clearwater, Joe, Laundrie, Nelson, Wavy, and Whitepine Lakes, to assess the historical and present day effects of these factors on zooplankton recovery. In this analysis we search for correlations between changes in the time series for zooplankton biomass, body length and species composition and changes in pH, metals, and fish.

Assessment of the potential for recovery of *Daphnia* species from copper and nickel impacts in soft water (PL)

N. D. Yan[1] and M. Celis Salgado[1]. [1]York University, Toronto, ON

Sudbury lakes were impacted by historical emissions of SO2 and metals. The acidity has fallen as result of liming and/or reduced emissions, but biological recovery has been slow and is still incomplete. Among the zooplankton, Daphniid (Crustacea, Cladocera) colonists have arrived, but most have failed to reestablish populations. One possible explanation is the lingering toxic effects of metals. Almost no assessments of metal toxicity exist for the Daphniid species native to the Canadian Shield. Bioassays based on the highest current copper and nickel concentrations in Sudbury lakes have been conducted with different *Daphnia* species, common inhabitants of the Canadian Shield lakes: *D. ambigua*, *D. pulex*, *D. pulicaria*, and *D. mendotae*, all collected from reference lakes in Ontario. Results indicate that copper and nickel mixtures are more toxic than the individual metals for the Daphniids under study and, that there is different sensitivity to the metals among the four species. The applied metal concentrations are triggering non-monotonic responses with minimum survival at mid-range concentrations. Considering a diminishing trend in the amounts of metals in the lakes, the higher mortalities in the middle concentration of those applied in this study suggest a critical threshold caused by the metals, which may prevent the recovery of the Daphniids in the restoration dynamics of the studied lakes.

The role of residual metals, predators, and contingency in the recovery of zooplankton communities (PL)

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Both the extent of impacts and the degree of recovery are often difficult to determine in areas that have suffered industrial damage. In the zooplankton of freshwater lakes, community structure is often determined by multiple, interacting physical, chemical, and ecological factors. Add to this complexity the variable effects of industrial stressors, as well as the exigencies of species re-colonization and the result is a patchwork of different zooplankton communities across the landscape that is not easy to interpret. One approach is to use multivariate analyses to dissect the effects of different factors. We analyzed a large data set of 87 lakes located in the area of Sudbury, Ontario. The lakes covered a range of industrial damage, as well as factors such as DOC, depth, productivity, and fish populations that are all known to affect zooplankton communities. Against a background of effects of productivity, lake depth, lake size, DOC, and urban influences, we detected lingering effects of industrial damage caused by low pH, residual metal contamination, and the ecological effects of predator (fish) extirpation. We further explored the implications of fish extirpation and unusual predatory regimes using enclosure experiments with the treatments: no fish, low fish biomass, and high fish biomass x presence/absence of the macroinvertebrate predator, *Chaoborus*. We found small but significant effects of both types of predators. Overall, our results suggest that zooplankton recovery from acidification depends not only on restored pH, but also on reducing metal levels, and restoring fish populations.

Climate-induced changes in lake thermal habitat alters predator-prey interactions in a recovering freshwater zooplankton community (PL)

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Recovery patterns of lakes from acid and metal deposition will interact with large-scale environmental stressors such as climate change. Biological interactions, as well as direct responses to changes in habitat quality, will affect the recovery process. The potential effects of climate change on spatially-occurring species interactions are poorly understood. We expect that climate-induced changes in lake thermal habitat will alter predation regimes, particularly in small, shallow lakes that are uniquely susceptible to warming. We used field enclosures to test the interactive effects of lake thermal habitat structure and predator type on avoidance behaviour and abundance in a recovering freshwater zooplankton community. We performed a factorial experiment with three levels of thermal habitat: "cool"-stratified, "warm" and "hot" –isothermal, and four predator treatments: control, surface-orienting Notonectidae, vertically-migrating *Chaoborus*, and both predators combined. Surprisingly, thermal habitat and predators had no effect on zooplankton depth distribution. We detected weak predator effects in the stratified habitat, and no predator effect in hot conditions. However, thermal habitat interacted with predator type such that *Chaoborus* significantly reduced zooplankton abundance only in warm isothermal conditions. The cost of vertical migration and compensatory dynamics between predators may reduce predation in stratified waters. In contrast, optimal temperatures and constant thermal conditions during vertical migration may increase the impact of *Chaoborus* in structuring the zooplankton community. However, there may be a threshold temperature where predators become heat- stressed and direct temperature effects structure the invertebrate community. This research addresses the role of biologically-mediated temperature effects in regulating zooplankton community recovery patterns.

Yellow perch (*Perca flavescens*) as a sentinel species for environmental effects monitoring in the Sudbury area (PL)

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Yellow perch (*Perca flavescens*) dominate Sudbury fish communities during early stages of recovery from acidification (pH>5). Predatory species begin colonizing as water quality improves (pH>6) but metal concentrations still exceed Provincial Water Quality Objectives in urban lakes near the smelters (<20 km). Yellow perch are useful sentinel fish species for environmental effects monitoring (EEM) because they have dietary shifts from zooplankltivory-to-benthivory-to-piscivory which are affected by abundance, trophic pathways, resource availability, and predation risk. We combined habitat use, life history, body shape, stable isotope, and diet analyses to assess whether yellow perch are able to adapt their morphologies in order to exploit pelagic and littoral habitats. Detailed biological measurements and meristic counts were made on yellow perch collected across a predation risk gradient of eight lakes (three lakes with no piscivores, two lakes with low piscivore abundance, and three lakes with high piscivore abundance). Yellow perch abandoned the pelagic zone in lakes with high piscivore abundances. After one year yellow perch abundance in an experimental piscivore transfer lake declined 75 % and after two years very few fish were found using the pelagic habitat. Stable isotope analysis revealed that yellow perch in the lakes with no piscivores or low piscivore abundance used food sources from both littoral and pelagic habitats while yellow perch in high piscivore abundance lakes utilized habitat specific food sources. Yellow perch responded to predation risk by increasing the dorsal spine length and body depth. Further biological recovery of Sudbury lakes can be assessed by monitoring changes in traditional EEM parameters such as abundance, as well as novel EEM parameters (i.e., body shape and foraging behavior).

The importance of benthic invertebrates for recovering food webs (PL)

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Benthic invertebrates are strongly exploited by fish and form a crucial part in the food webs of freshwater lakes. In the Sudbury area, benthic invertebrates seem to have a reduced biomass and diversity, which may hamper biological recovery. This energetic bottleneck should be especially restricting in lakes with high levels of piscivory, within which yellow perch (*Perca flavescens*) primarily use the littoral zone. We studied perch in two lakes with low piscivore abundance and two with high piscivore abundance over a two year period. Additionally, in 2006 one of the low piscivore lakes was stocked with 184 smallmouth bass (*Micropterus dolomieu*). We measured perch use of pelagic and littoral habitats, determined diet and stable isotope signature, and compared these to resource availability (i.e., benthic invertebrate biomass). High piscivory reduced overall perch biomass and caused a shift in habitat use from the pelagic to the littoral that was reflected in the stable isotope signature. There was little variation in perch carbon signature in low piscivore lakes while in high piscivore lakes there was high variation and a greater use of littoral carbon sources. Chironomids were most abundant both in the environment and in littoral perch diets in all lakes. While total benthos biomass was similar in all lakes, larger benthic invertebrates (Odonata and Trichoptera) were more abundant in lakes with abundant piscivores. The arrival of piscivorus fish seems critical for recovery as it not only changes the population size structure and behaviour of the perch but also affects the benthos.

Use of littoral benthic invertebrates to assess factors that delay biological recovery of acid and metal damaged lakes (PL)

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Biological recovery of aquatic ecosystems from acidification damage is proving to be a slow process in lakes near the nickel-copper smelters in Sudbury, Ontario. The delays are presumably because of persistent confounding factors such as residual metals, habitat damage, altered predator/prey interactions and other multiple ecological stressors. Assessments of benthic invertebrate communities in 24 Sudbury and Killarney Park lakes were conducted using rapid bioassessment methods developed by the Ontario Ministry of the Environment. At the time of sampling, all lakes were chemically recovered ($pH > 6.0$) from acidification but spanned a wide range of variables, including time since reaching pH 6.0, waterborne Cu and Ni concentrations, percent organic matter in lake sediments, littoral habitat, and littoral fish species biomass. Partial redundancy analyses with four environmental variable groups (water chemistry, fish communities, physical lake descriptors, and littoral habitat) showed that fish community variables explained the most variance in abundance, percent composition, and presence-absence data for littoral benthic invertebrates (23 %, 24 %, and 24 %, respectively). Specifically, fish species richness explained significant ($p < 0.05$) amounts of variance in all analyses. Only abundances of littoral benthic invertebrate taxa were also significantly ($p < 0.05$) explained by chemical or habitat variables. Invertebrate percent composition and presence-absence were also significantly explained by physical lake descriptors. Results from this study suggest that remediation techniques such as manipulation of predator/prey interactions through fish introductions would be the best tools for recovery of littoral benthic invertebrates. However, complexities in interactions of environmental variables groups should still be investigated.

The role of land reclamation and forest regeneration on the recovery of near-shore benthic invertebrate communities (PL)

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Watersheds with well developed vegetation and soil layers help to regulate hydrology and influence stream chemistry, and provide an important source of organic material for the biotic communities in the receiving waters. The denuded landscapes of the Sudbury area are likely hindering the natural recovery process of the benthic invertebrate communities in the lakes. It is hypothesized that correlations exist between metrics of benthic community health (family abundances, taxa richness, and evenness) and metrics of forest health of associated catchments (tree density, canopy cover, species richness) as well as the condition of the forest soil. This study makes use of three lakes along an existing gradient of landscape denudation that resulted from the Coniston smelter, and includes a treated sub-catchment from a land-regeneration liming experiment in the mid 90s. Sub-catchments and the associated streams, along with the stream-lake confluence zones (deltas) define 15 sites that span the three lakes. Ordination analyses reveal relationships between soil condition, stream water quality, metrics of forest health, and benthic invertebrate community composition along the gradient. By constructing models that connect the health of near-shore biota to the health of the surrounding watershed, best-practice restoration strategies can be developed that work towards the recovery of damaged lands and their receiving waters.

PESTICIDES AND OTHER AGRICULTURAL STRESSORS

Effects of a ternary agricultural insecticide mixture on two aquatic invertebrates (PL)

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Although numerous studies have investigated the lethal effects on benthic invertebrates of exposure to a single pesticide, lethal exposures to single chemicals do not necessarily reflect real-world environmental conditions. Ambient pesticide exposures in agricultural catchments often contain low concentrations of pesticide mixtures; therefore it is important to investigate sublethal responses of organisms, along with lethal responses. Our objective was to investigate the single and interactive effects of three insecticides (chlorpyrifos, dimethoate and imidacloprid) that are commonly used in potato production in New Brunswick on two aquatic insects, *Chironomus tentans* and heptageniid mayflies. We examined this question using acute (96 h – chironomid and mayfly) and chronic (28 d - chironomid) laboratory-based bioassays, where various endpoints were measured (e.g. survival, larval growth, emergence rate and size at emergence). These data indicate that dimethoate is considerably less toxic than chlorpyrifos and imidacloprid, and also that the differences in lethality between individual, binary and ternary mixtures are small. The interactive effects of the insecticides at lethal and sublethal exposures on the measured endpoints exhibit a range of responses including additivity and antagonism. The ability of this research to help provide a better understanding of the impacts of agricultural pesticide application on aquatic systems will be discussed, along with implications for pesticide regulation given that pesticide impact may be underestimated by individual chemical toxicity studies.

Effects of chronic exposures to the herbicides atrazine and glyphosate on larvae of the three-spined stickleback (*Gasterosteus aculeatus*) (PL)

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Atrazine and glyphosate are two herbicides widely used in Canadian agriculture. Since 2002, Fisheries and Oceans Canada and the Pest Management Regulatory Agency of Health Canada have evaluated impacts of pesticides on marine and freshwater ecosystems. We selected the three-spined stickleback (*Gasterosteus aculeatus*) as a sentinel species for the Saint-Lawrence estuary, since it reproduces in sensitive coastal habitats during the summer, the peak season of herbicide use. In addition, sticklebacks are a good model for effects of both estrogenic (vitellogenin, VTG) and androgenic (the male nest-protein spiggin) contaminants. Stickleback adults from a clean reference site were allowed to reproduce in the laboratory and the fertilized eggs were incubated until hatching. Larval sticklebacks (<24 h old) were exposed for 42 days to four concentrations (0.1, 1, 10 and 100 $\mu g \cdot L^{-1}$) of each herbicide and to positive controls ethinylestradiol (0.05 $\mu g \cdot L^{-1}$, EE2) and dihydrotestosterone (3 $\mu g \cdot L^{-1}$) for estrogenic and androgenic effects, respectively. The survivors were measured (length and wet weight) then conserved for analyses of reproductive biomarkers (intersex, VTG, spiggin). Larval survival was 100 % in both herbicide exposures. There were no significant effects on growth in glyphosate exposures, while mean larval growth was significantly reduced at atrazine concentrations of 1, 10 and 100 $\mu g \cdot L^{-1}$ ($P < 0.05$) and showed a slight dose-response. There was no induction of VTG in larvae exposed to either herbicide, yet EE2 exposure caused a significant induction of VTG, ranging from 123 $\mu g \cdot g^{-1}$ to 10.4 $mg \cdot g^{-1}$. Analyses of intersex and spiggin are in progress.

Population indices and reproductive biomarkers of Saint Lawrence Estuary three spine sticklebacks (*Gasterosteus aculeatus*) subjected to inputs of agricultural chemicals (PL)

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Pesticides are widely used in the Saint Lawrence Estuary (SLE) watershed and runoff after storm events may affect spawning and feeding areas used by marine fish species. We sampled stickleback populations from four sampling sites, two agricultural - Trois-Pistoles (TP) and Isle Verte (IV), an urban site (PP) and a reference site (Bic) with the principal objective of determining effects on reproduction. In 2002-2004, water samples for chemical analyses were also collected from tributary rivers during periods of heavy pesticide application. Levels of pesticides were low, with highest levels at TP and IV. Atrazine and desethylatrazine in these habitats derive from the St Lawrence River (e.g. fluvial transport) and from the inflowing rivers, while the main sources of simazine and metolachlor are the tributaries. Sticklebacks were measured (length, weight, sex ratio, GSI, HSI) and reproduction biomarkers (gonadal histology, plasma vitellogenin) evaluated. Females had normal (elevated) levels of VTG during the breeding season; plasma VTG in male fish was low but measurable. Females from IV showed a high proportion of atretic oocytes, suggesting reproductive dysfunction. The IV site receives runoff containing several pesticides, including atrazine, desethyl-atrazine, simazine and metolachlor, as well as agriculturally applied nutrients. Sampling in 2004 and 2006 confirmed the dysfunction at IV and indicated low dissolved oxygen levels and inputs of nutrients as possible additional stressors. Risk assessments of pesticides must take into account other environmental stressors, such as excess levels of nutrients from fertilizers and/or extreme physical-chemical conditions associated with tidal zones.

Effects of ammonia on fish and shellfish brain cell lines (PL)

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Hyperammonemia is detrimental to brain function and has been studied almost exclusively in mammals, although this is interesting because hyperammonemia is becoming a common condition in aquatic environments but effects in fish or shellfish neural cells have been seldom studied. *Ex-vivo* culture of nervous tissue cells from various organisms has proven useful in several areas of research including aquatic toxicology and neurobiology. Cell cultures are used to study the functions of neurons and glial cells and also to test potential neurotoxicant compounds. However, freshly isolated cultures of neural cells are cumbersome to prepare, time consuming and costly. Established cell lines derived from neural tissues have proven useful in many areas of research and have been used as models for neural regeneration studies as well as for elucidating mechanisms of neurotoxicity and neurophysiology. In this study, we report on the use of established cell lines derived from brain tissues of crayfish (*Orconectes limosus*), goldfish (*Carassius auratus*), rainbow trout (*Oncorhynchus mykiss*), and American eel (*Anguilla rostrata*) exposed to varying concentrations of NH_4Cl at various time points and evaluated various cellular responses including viability, membrane integrity, lysosomal function and general cellular morphology. These cells were tested to examine if there are interspecies differences in their tolerance to ammonia since goldfish and eels are fairly tolerant to hyperammonemia *in vivo*.

Effects of pesticides or their formulation on the amphipod *Corophium volutator* (PO)

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The exposure of marine organisms to pesticides can take place in water or sediments. Exposure to a formulation will include the presence of surfactants and trace contaminants along with the active biocide and could lead to synergistic or antagonist effects relative to a single component. If these compounds arrive in an estuary, they could potentially have adverse effects on amphipods. The survival, behaviour, growth, swimming and/or lipid content of ***Corophium volutator*** were examined in two day exposures to spiked sediments or seawater at levels ranging from 0.1 to 1,000ng·g^{-1} or ng·mL^{-1}. Bravo, Lorsban, and Roundup containing the herbicides or insecticides chlorothalonil, chlorpyrifos and glyphosate, commonly applied in Prince Edward Island, were used in the experiments. Combining the present results with chemical analyses of sediments and seawater, along with the abundance and diversity of inter-tidal benthic communities will ultimately help assign a cause to changes observed in multi-disciplinary field studies.

Effect of herbicide mixtures on microbial communities from prairie wetlands: a mesocosm approach (PO)

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Wetlands in the prairie pothole region (PPR) are regularly interspersed among intensive agricultural operations where herbicides are commonly used. Herbicides entering wetlands may have direct and indirect effects. The main objective of this study was to determine the response of wetland microbial communities to a range of environmentally relevant concentrations of mixtures of the seven most prevalent herbicides found in prairie aquatic ecosystems. In late May 2007, a wetland mesocosm experiment was set up at St. Denis National Wildlife Refuge, 45 km east of Saskatoon, SK, CAN. Eight holding tanks were positioned adjacent to Pond 79. Herbicide mixtures of MCPA, clopyralid, dicamba, dichloroprop, mecoprop, 2, 4-D, bromoxynil, and glyphosate at 1, 500 and 1000 times the environmentally occurring concentrations, were added to each tank. Attached algae and bacteria (biofilms) were harvested on days 8, 14 and 21, while free-living microbial communities were sampled at 1, 2, 3, 7, 14 and 21 days post-treatment. Samples were taken for bacterial and primary production, bacterial and algal biomass, biofilm bacterial diversity, algal protein to carbohydrate ratios, and water chemistry parameters. Preliminary results indicate an initial decline in primary production, bacterial production and sestonic protein to carbohydrate ratios with recovery noted from 14 to 21 days after treatment at 1 and 500 times.

Could insects in neuston act as vectors for pesticide exposure of lobster postlarvae in the nearshore marine environment? (PO)

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Previous studies in the Southern Gulf of St. Lawrence have shown that there is potential for movement of pesticides into coastal marine waters. However, the pathways and likelihood of such an occurrence causing measurable effects remain to be determined. One consideration is the potential for dietary exposure of postlarval American lobster *(Homarus americanus)* through ingestion of contaminated insects. Insects are a common item in the diet of postlarval lobsters. Moreover, insects are often present in neuston samples, particularly in summer, when extensive pesticide treatments are underway and postlarval lobster are present in nearshore waters. Summer storms after extended dry periods may enhance pesticide runoff and increase fluvial contribution to the neuston. In July and August 2007, neuston and sea water were sampled within and off the mouths of several estuaries. These samples were analyzed for a suite of high use, high risk base-neutral pesticides that are used on adjacent land. If these pesticides are present in the nearshore neuston and become part of the lobster diet, the potential for negative biological effects exists.

TOOLS TO ASSESS TOXICITY AND BIOAVAILABILITY IN SUPPORT OF RISK ASSESSMENTS

The effect of environmental ligands on toxicity of nickel to *Lemna minor*

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Abstract

The Biotic Ligand Model (BLM) approach, now incorporated in the regulatory framework for copper by the US EPA, is well-developed for predicting toxicity in fish and has been extended to other aquatic organisms such as invertebrates and algae. However, little information is available for BLM's application to aquatic plants such as *Lemna minor*. *L. minor* is used to monitor mine effluent quality under the Metal Mining Effluent Regulations (MMER) and is sensitive to trace metals commonly discharged in mine effluents such as nickel. In the present study, the effect of environmental ligands, such as dissolved organic carbon (DOC) and flotation ligands, on nickel toxicity to *L. minor* were examined to develop a bioavailability model for mine effluent and receiving waters. The flotation ligands commonly used by mining industries in Canada include diethylenetriamine (DETA) and triethylenetriamine (TETA). The effect of DOC on nickel toxicity was relatively small (less than a factor of 2) and can be explained solely by complexation of Ni^{2+} with DOC. Alternatively, the protective effect of DETA and TETA on Ni toxicity was much less than expected based on estimated complexation. The results of this study will be used to develop a bioavailability model for *L. minor* toxicity which can be used for interpretation of MMER monitoring data and for investigation of cause.

Introduction

The ability to accurately predict the toxicity of a metal is being recognized as an important tool for establishing relevant water quality guidelines and for regulating industrial operations. The US EPA has adopted the Biotic Ligand Model (BLM) as the regulatory tool to establish water quality guidelines for copper (USEPA, 2007). The BLM is a model that predicts site-specific metal toxicity to an organism, considering metal complexation and speciation in solution surrounding the organism. Metal speciation is defined as the occurrence of a metal in separate identifiable forms, where the free metal ion is generally accepted as the most bioavailable form. The BLM also takes into account interactions between the metal ions and competing cations at the binding sites on the organism-water interface (Lock etal. 2006) The BLM has been successfully applied for fish and invertebrates, but seems to be less applicable for plants as the initial binding site (the root cortex) is not typically the primary site of toxicity (Antunes etal., 2006)

The aquatic plant *Lemna minor* is one of the most sensitive species used in Metal Mining Effluent Regulations (MMER) (Vigneault et al., 2005). *L. minor* is an aquatic plant that is sensitive to trace metals (such as Ni) commonly discharged in mine effluents (Vigneault et al., 2005). The prediction of nickel toxicity in *L. minor* has been previously attempted using cross-species extrapolation of existing nickel BLMs. The results show that the nickel toxicity data for *L. minor* was better explained by the *Daphnia magna* BLM rather than the algae BLM (Nickel Producers Environmental Research Assoc. 2007). This highlights the importance of a model tailored for plants and the need for examining the effect of environmentally relevant ligands on nickel toxicity to *L. minor*, which has rarely been done.

The only organic complexant currently included in the BLM is dissolved organic carbon (DOC). However, flotation reagents are often used in the mining industry for wastewater treatment and are strong metal complexants. Flotation agents commonly used in Canada include diethylenetriamine (DETA) and triethylenetriamine (TETA). No studies in the existing literature examine the toxicity of metals in the presence of DETA or TETA. However, some studies have looked at the toxicity and bioavailability of metals in the presence of xanthates which are also commonly used as flotation ligands (Boening, 1998, Block and Part, 1986, Block, 1991) Xanthates are known to form hydrophobic compounds with metals (Boening, 1998) In fact, studies have shown that the presence of sodium isopropyl xanthate increases absorption of cadmium by fish gills (Block and Part, 1986, Block, 1991) As a result, bioavailability may not be described by the BLM framework, which considers the free ion, only, as the active metal species at the biological interface.

The BLM approach would be a useful tool for explaining Metal Mining Effluent Regulations (MMER) data because it allows prediction of metal toxicity based on estimates of metal speciation (Paquin et al., 2002) The present study aims to test the BLM hypothesis that IC25 levels expressed as free nickel activity ($\{Ni^{2+}\}$) are constant, regardless of the complexant(s) in the exposure solution. This will be attempted by studying the effects of DOC and flotation ligands on Ni toxicity. A mechanistic predictive model for *L. minor* will be relevant for regulation of mining effluents and receiving waters since the plant is commonly used as a standard test organism for regulatory purposes. The long-term objective of this research is to develop a predictive model for plants which includes flotation agents. Such a model will be beneficial for specifically considering metal bioavailability in Environmental Risk Assessment and Water Quality Guidelines.

Methods

Lemna minor (common duckweed) is a floating vascular macrophyte. It is suitable for metal biomonitoring because it is ubiquitous and stationary, small, easy to identify and sensitive to metals (Blinova, 2004). The present study followed the standardized Environment Canada protocol (Environment Canada, 2007) which involves a 7-day test. The culture medium was modified Hoagland's E^+ and the test medium was modified APHA (with added 0.5 $mg \cdot L^{-1}$ of DOC and exclusion of EDTA). EDTA was excluded due to its ability to complex the test metals; however, its exclusion can reduce availability of nutrient metals to the plant through precipitation. Thus, DOC was added as a buffer to prevent nutrient metal loss at pH 8 due to precipitation and adsorption. Preliminary study showed no effect of DOC at this concentration on nickel toxicity to *L. minor*. The standard test endpoints are frond count (FC) and dry weight (DW). The present study additionally examined root length (RL) and surface area (SA, using a LemnaTec image analyser). The DOC used throughout this research was Suwannee River dissolved organic matter (International Humic Substances Society, St. Paul, Minnesota). The flotation reagents tested were diethylenetriamine (DETA) (Fluka, Netherlands) and triethylenetriamine (TETA) (Fluka, Switzerland). All ligands were tested at environmentally relevant concentrations. The nickel concentrations used in the analyses were nominal. Windermere Humic Aqueous Model (WHAM) VI (Tipping, 1998) was used to estimate {Ni2+} with modified parameters (Van Laer et al. 2006). For all tests, 25 % inhibition concentrations, IC25, were estimated using the Comprehensive Environment Toxicity Information System (CETIS version 1.025B, Tidepool Scientific Software, 2004). The nominal Ni_{Tot} concentrations tested in the bioassay were 0, 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 $\mu g \cdot L^{-1}$. The 25 % inhibition concentrations based on $\{Ni^{2+}\}$, $IC25Ni^{2+}$, were estimated using $\{Ni^{2+}\}$ in solution calculated by WHAM VI. Data entered into WHAM VI include the bioassay nominal Ni_{Tot} concentrations, pH, ligand concentration, as well as the cation and anion concentrations of the APHA test media. Note that due to the slow equilibrium kinetics of Ni in natural waters (Gopalapillai et al. 2008), test solutions were made 3 days prior to test initiation.

Results and Discussion

Preliminary studies showed root length to be the most sensitive and least variable endpoint. Thus, toxicity based on inhibition of root growth was used to examine the effect of ligands on nickel toxicity. WHAM VI calculations predicted significant complexation of nickel by DOC. In the presence of 20 mg·L^{-1} of DOC, only 7 % of a [Ni$_{Tot}$] of 15.6 µg·L^{-1} remained as Ni^{2+}. As a result, the expected effect of DOC was a reduction in toxicity of nickel (IC25$_{NiTot}$). However, the toxicity reduction at this DOC range was relatively small (less than factor of 2), much less than the reduction in Ni^{2+} would suggest (Figure 1).

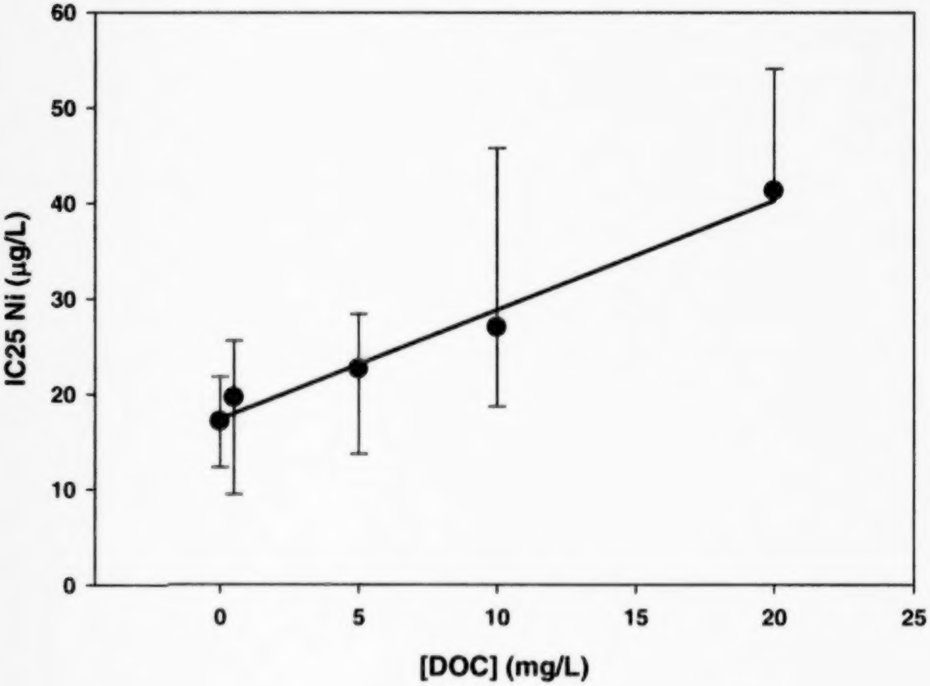

Figure 1. Effect of DOC on Ni toxicity to *Lemna minor*. Toxicity is represented as total Ni (IC25$_{NiTot}$). Error bars represent 95 % confidence interval.

To test the BLM hypothesis that IC25 levels expressed as free nickel activity are constant (i.e. effect of ligands can be explained solely by complexation), the growth inhibition data were plotted against WHAM VI calculated {Ni^{2+}}. As expected by the BLM framework, the relationship between DOC concentrations and IC25$_{Ni2+}$ was observed to be constant (i.e. as [DOC] ranged from 0.5 mg·L^{-1} to 20 mg·L^{-1}, IC25$_{Ni2+}$ was 10.9 ± 3.3 µg·L^{-1}). Thus, the effect of DOC on Ni toxicity can be modelled using the BLM framework.

Studies on the effects of flotation ligands on nickel toxicity showed that the presence of 10.3 mg·L-1 of DETA reduced Ni toxicity (IC25$_{NiTot}$ 113 µg·L^{-1}) with respect to the control (IC25$_{NiTot}$

19.1 ± 0.6 $\mu g \cdot L^{-1}$). Similarly, the presence of 14.6 $mg \cdot L^{-1}$ TETA also reduced Ni toxicity ($IC25_{NiTot}$ 88.5 $\mu g \cdot L^{-1}$). Most likely, the observed reduction in toxicity was due to a reduction in $\{Ni^{2+}\}$ (i.e. complexation). However, WHAM calculated negligible $\{Ni^{2+}\}$ in the presence of DETA (maximum 10-13 $\mu g \cdot L^{-1}$ of Ni^{2+}) and TETA (maximum 10-15 $\mu g \cdot L^{-1}$ of Ni^{2+}) based on complexation (log K_{NiDETA} is 10.5, log K_{NiTETA} is 14.0). As a result, the BLM framework will predict a much larger reduction in toxicity than was observed. Table 1 compares the measured and predicted $IC25_{NiTot}$ in the presence and absence of the various environmental ligands. Predictions of $IC25_{NiTot}$ in the presence of DETA and TETA were nearly 2 orders of a magnitude higher than the measured values. Note that DETA and TETA controls (ligand only) showed no significant toxicity to *L. minor* (data not shown). Thus, it is likely that the effect of flotation ligands on the toxicity of nickel to L.minor presents an exception to the BLM-expected behaviour. However, further research is necessary to confirm these findings.

Table 1. Comparison of measured versus predicted $IC25_{NiTot}$ for *Lemna minor* in the absence and presence of environmentally relevant ligands.

LIGAND	**[LIGAND] ($mg \cdot L^{-1}$)**	**Measured IC25 Ni_{Tot} ($\mu g \cdot L^{-1}$)**	**Predicted IC25 Ni_{Tot} ($\mu g \cdot L^{-1}$)**
CONTROL (NO LIGAND)	0	17.1 (12.3 - 21.8)[a]	24.1
DISSOLVED ORGANIC CARBON	0.5 – 20	27.6 ± 9.8 [b]	33.5
DIETHYLENETRIAMINE	10.3	113 (70.0 – 426)	4.23 x 103
TRIETHYLENETRIAMINE	14.6	88 (N/A – 214)	5.88 x 103

[a] Range between lower confidence limit and upper confidence limit

[b] Standard deviation for a minimum of n = 3

In conclusion, the effect of DOC on Ni toxicity to *L.minor*, although small, can be explained by complexation alone, and can thereby be modelled using BLM. However, the effect of flotation ligands on Ni toxicity was much less than expected based on estimated complexation. As a result, this possible non-BLM type behaviour requires additional research to understand the underlying mechanism of toxicity. This leads us to the next steps in this research, which will be to explore the possibility that Ni complexed to flotation ligands (including xanthates) are bioavailable. In addition, methods will be developed to measure metal speciation in solution as well as ligand concentrations.

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Assessment of single extraction procedures for quantifying the bioavailable metal fractions in sediment (PL)

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Methods such as porewater and solid-phase extractions have been used to isolate and quantify the potentially bioavailable fraction of metals in sediment. The objective of this study was to determine whether metal concentrations in sediment porewater, isolated using an *in-situ* dialysis sampler (mini-peeper) or by centrifugation, or whole-sediment extractions, using single chemical extraction methods, better correlated with benthic community effects than whole-sediment total metal concentrations. In preliminary experiments, porewater sampling and single extractions of field-collected sediment from a site near a uranium operation in northern Saskatchewan were completed to identify the extraction methods which appeared to best identify the bioavailable metal fraction. The initial reference point for the bioavailable metal fraction was assumed to be metals in sediment porewater collected using mini-peepers. The extractants evaluated in this experiment were purified water, 1 M $MgCl_2$, 0.11 M KH_2PO_4, 0.5 M EDTA, and 1 M HCl. Extraction methods that yielded metal concentrations slightly higher and substantially higher than porewater metal concentrations, for the majority of the metals evaluated, were chosen to further investigate how these metal concentrations would correlate with benthic community effects *in-situ*. Results from this study aim to provide further evidence that "bioavailable" metal fractions in sediment, rather than total metal concentrations, are more accurate for correlating metal exposure with benthic community effects in metal contaminated sediment.

Biological and chemical measures of bioavailability compared to the results of chronic toxicity tests (PL)

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The soil contact exposure pathway for ecological receptors can be the main driver of ecological risk assessments. There is currently no standard method to measure directly the bioavailability of contaminants in soil to ecological receptors. Bioavailability of contaminants to soil-dwelling organisms can be drastically different among sites with soils that vary with respect to their physico-chemical characteristics and contamination levels. Therefore, in order to accurately measure bioavailability, tools tailored to the contamination and soil type at the site must be used that account for site-specific contaminants and soil characteristics. Field soils were obtained from a metal-contaminated site in Toronto, ON. Concentrations of Pb, Cu, Zn, and Cr in the soils exceed invertebrate and plant benchmarks commonly employed in ecological risk assessments. The bioavailability of metals in these soils was measured using biological methods including a 21-day earthworm bioaccumulation test and a battery of chronic toxicity tests using plants and invertebrates. Samples of exposed earthworms were taken during the 21-day bioaccumulation test for the determination of metal concentrations in worm tissue, and kinetic uptake and elimination rate constants and bioaccumulation factors (BAF) were determined where appropriate. Chemical measures were used to assess metal bioavailability, including calcium chloride and cyclodextrin extractions. In addition, metals in the soils were extracted using a novel Simulated Earthworm Microbial Extraction Device (SEMED). The four measures of bioavailability (i.e., bioaccumulation, calcium chloride, cyclodextrin, and SEMED tests) were then compared to the results of the toxicity tests.

Expanding the applicability of boreal forest plants for assessment of soil contaminants using the wetland plants, cattail and bulrush (PL)

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Development of a standardized soil toxicity test method using boreal forest species has focused on upland plants. However, wetland regions comprise approximately 16 % of the land area of Canada. Therefore, inclusion of ecologically relevant wetland species to the existing test battery will widen the applicability of the plant growth inhibition test, providing useful information to support location, description and reclamation of contaminated wetland sites. Five species of bulrush and two species of cattail were investigated with the intention of using these species in the method for upland plants. Since seed of bulrush is dormant when purchased, methods of stratification and scarification were tailored to each species to achieve germination of at least 50 %. This allows planting of young seedlings in test soils. Cattail seed was found to require freezing, either outdoors or in the lab. Growth of week-old seedlings was assessed in artificial soil and in three natural reference soils in tests of 4 weeks duration. Survival to test end was >80 % for seedlings of all species, even though soils were maintained at lower moisture levels derived for maintaining soil structure in testing with upland boreal plants. Vigorous growth of shoots and roots was observed in three of four control soils, the exception being a podsol from Ontario low in pH. These studies indicate that wetland plants have potential to be incorporated into a future test method measuring early seedling growth using boreal forest plant species.

Toxicity assessment of remediated soils: Alberta's Tier 2 approach (PL)

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Alberta Environment has recently published Alberta Tier 1 and Tier 2 soil and groundwater remediation guidelines. In many cases, Tier 1 guidelines for petroleum hydrocarbons (PHCs) in soil are not applicable for sites where PHCs have been weathered or aged over a prolonged period. Reduced bioavailability of PHCs in soil to ecological receptors through the soil-contact exposure pathway is typically observed in these contaminated soils. Alberta Environment has recently released a draft protocol for the development of Tier 2 eco-contact guidelines for PHC-contaminated soils. In order to achieve regulatory closure for a site, proponents must demonstrate that the residual PHC concentrations in soil are not toxic. If soils are deemed non-toxic to the minimum required test battery, then regulatory closure may be achieved; however, there are established criteria that must be met. Three case studies are presented where ecotoxicity data were generated for PHC-contaminated soil and then subjected to the Tier 2 Pass/Fail program. PHC-contaminated soils from the first two case studies were extensively bioremediated but residual levels still exceeded Tier 1 guidelines; the soils from the third case study were contaminated with both metals and weathered PHCs, and had elevated salinity levels. The ecotoxicity data from the first study passed the Tier 2 criteria; however, the influence of soil physico-chemical characteristics on test species performance was significant. The second case study involved soils contaminated with PHCs at three different concentrations; the lowest level passed the Tier 2 criteria whereas the two highest levels failed. In the third case study, the soils passed the assessment. The applicability and limitations of the draft Tier 2 Pass/Fail program based on the results of these case studies is discussed.

Evaluation of commercial toxkits in comparison to standard environmental acute toxicity tests (PO)

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Environmental toxicity is measured through standardized acute toxicity tests such as those realized with water fleas (*Daphnia magna*), green algae (*Pseudokirchneriella subcapitata*, previously called *Selenastrum capricornutum*) or bacteria (*Vibrio fisheri* via Microtox). The first two require breedings that may be difficult to set up and time-consuming to maintain. Since a few years ago, toxkits are now offered on the market. Those toxkits are presented as being faster and less expensive than standard toxicity tests. The main difference between toxkits and classical toxicity tests is that kits contain all that is necessary to realize the test, including organisms under dormant form that are reanimated right before the test. This study aimed to compare the relative sensitivity and variability of 3 toxkits (Daphtoxkit, Rotoxkit and Thamnotoxkit) with standard environmental toxicity tests (Microtox, daphnia, and green algae tests) using two reference toxics: a mixture of seven heavy metals (as an inorganic pollutant) and a mixture of chlorophenol and resin acid (as an organic pollutant). Results indicated that toxkits are relatively simple to use and have sensitivity and variability comparable to that of standard tests. Moreover, almost all tests (i.e. including toxkits but not Microtox) show higher sensitivity to inorganic pollutant than to organic pollutant. However, it appears that toxkits are not really less expensive than standard tests because each toxkit includes only the material to realize 6 assays, which is often insufficient when pre-tests are necessary to evaluate the dilution range needed or when tests have to be redone for various reasons.

Demonstration of a planar optode-based system for evaluating the effects of contaminants and other stressors on benthic communities and processes (PO)

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The use of transparent planar optodes for dynamic measurements of dissolved oxygen (DO) and pH in sediment profiles provides a new approach for the assessment of the effects of environmentally realistic concentrations of contaminants on microbial, meiobenthic and macrobenthic communities. Planar optodes are sheets of acetate lined with different fluorosensors excitable at specific wavelengths, which reversibly bind to the chemical of interest (O_2, H^+), providing two-dimensional imagery of the distribution of such chemicals in a sediment profile. This allows the visualization of these parameters in full two-dimensions, corresponding directly to visible high-resolution sediment profile images, reflecting a variety of aspects of sediment and benthic community dynamics. Narrow environmental testing chambers following the "ant farm" model, with one clear and three black walls, were constructed for the assessment of the effects of tube-dwelling and burrowing benthic organisms on sediment pH and DO in the presence and absence of contaminants. Planar optodes will be attached to the inner face of the clear wall, and the test chambers will placed in a specially constructed black box with electronic components for digital time-lapse photography of sediment pH and DO profiles under a variety of environmental conditions. Results of laboratory tests performed to establish optimum lighting conditions and calibrate the images to actual pH and DO measurements will be presented.

PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

The Municipal Wastewater Perspective on Pharmaceuticals and Personal Care Products

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The presence of trace levels of pharmaceuticals and substances common in personal care products in surface waters and drinking water is a matter of growing concern in our society. Reports of their presence and effects are no longer restricted to scientific literature, but have been the subject of headlines in the popular media for a number of years now. Much of the focus has been on the endocrine-disrupting characteristics of these substances. While there have been no definitive correlations with human health effects, there is a growing body of evidence of effects in aquatic organisms which would be typical of exposure to such substances.

Effluents from municipal wastewater treatment plants are often linked to observed effects in the environment. However, it is not the municipal wastewater sector which generates these substances. Society has generated the demand for these substances as they are perceived to improve our quality of life: they are our medicines, our cosmetics, our cleaning products. They are released into municipal wastewater systems by citizens and industrial, commercial and institutional (ICI) customers served by municipalities. Municipalities are charged with treating our water-borne wastes and are often seen as responsible for these discharges.

Current Municipal Wastewater Treatment Capabilities

The challenges municipalities face extend far beyond PPCPs and include numerous other chemicals received by municipal wastewater treatment plants at trace levels. These are now commonly referred to as "microconstituents" by the sector. The current generation of wastewater treatment plants was never designed specifically to deal with such substances. Collecting human wastewater began as a means to reduce human exposure to pathogens responsible for diseases at epidemic levels such as cholera, which still represent problems in developing countries. Treatment began to mitigate the problems collection created due to the spatial concentration of discharges from the collected wastewater. Treatment plant design was focused on conventional aggregate parameters such as biochemical oxygen demand (BOD) which deletes dissolved oxygen available to organisms in water and "total suspended solids" (TSS) which cloud the water and can affect fish habitats. Only in the last few decades has the focus begun to consider the effects of nutrients causing eutrophication, also related to dissolved oxygen, and disinfection, in a return to concerns about pathogens as population growth and higher densities increase chances of exposure.

Municipal wastewater collection and treatment involves massive infrastructure. The ability to treat wastewater has been limited by the technical difficulties and costs related to treating the large volumes of water involved. The timescales required to change infrastructure are long. The potential problems related to PPCPs have only become evident only relatively recently on this time scale. These substances have been in use much longer than we have been able to detect their presence at trace levels with new laboratory analytical technologies and begin to understand their effects with, for example, advanced histological techniques. Correlations have been facilitated by the significant advance in computing technologies in the last few decades.

These conventional means of wastewater treatment, however, are able to provide some treatment for these "new" substances of concern. Secondary treatment typically involves settling of solids, both inorganic and organic, and biodegradation of organics which have not settled or are dissolved in the wastewater. PPCP substances can adsorb to the solids which are separated and can be biodegraded. However, more complex organics' molecules are often more difficult to biodegrade. Processes which have been developed for other reasons, such as biological nutrient removal, can create conditions which lead to better biodegradation. The microorganisms which biodegrade organic substances are collected near the end of a secondary process and are recirculated into the beginning of the process to ensure an adequate abundance to achieve the desired treatment. The resulting "solids retention time" (SRT) creates an average age for the biomass in the order of days. One theory is that this allows the biomass to acclimate to the substances they are exposed to and adapt to the more complex substances so they can use them as a food source. This also increases the adsorption of these substances onto solids, partitioning them into the wastewater sludge stream. This can lead to issues with the some uses of treated sludges, commonly referred to as "biosolids". While an important issue, discussion of biosolids is beyond the scope of this paper.

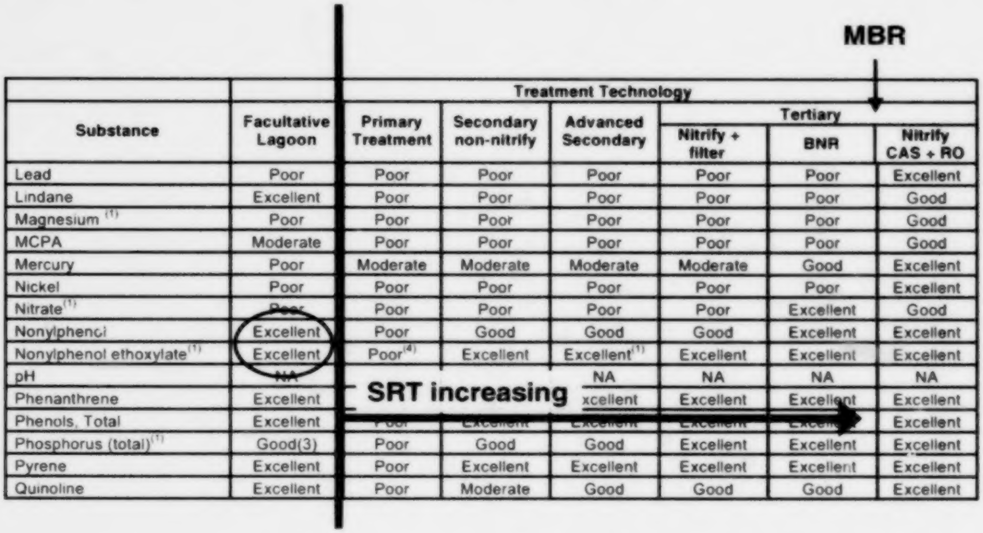
New Opportunities for Treatment

The municipal wastewater sector has begun to turn its attention to concerns about microconstituents in light of the new societal concerns. The abilities of conventional and new processes to deal with them are being increasingly studied and there are positive signs. Conventional activated sludge processes to remove BOD typically have an SRT of about 5 days. Modifications of this process to effect "biological nutrient removal" (BNR) use longer SRTs. Ammonia removal, in particular, requires a minimum SRT of about 10 days. It has been found that these longer SRTs also provide much better removal of more complex organics. Such changes can be made to conventional processes, but "de-rate" their capacity to deal with the volumes of wastewater received, thus requiring expansion of facilities in addition to the modifications needed to increase the biomass recirculation necessary to achieve longer SRTs.

New processes to deal with disinfection and taste and odour problems, such as advanced oxidation through the use of ultraviolet irradiation and/or ozonation have also been found to destroy PPCP substances, although operating costs are very high. Processes employing membrane bioreactors (MBR) use even longer SRTs and membranes with very fine pore sizes such as those used for nanofiltration and reverse osmosis (RO) can even provide a physical barrier to these substances, the pore sizes being smaller than the molecules themselves. However, with every advance comes significant technical and engineering challenges related to applying the technologies to the large volumes and "dirty" matrix wastewater represents. For example, the micro-organisms inherent in wastewater and used in biodegradation stages tend to foul membranes. New processes may also generate new concerns such as undesirable by-products, which need to be identified.

Table 1 presents the relative abilities of different treatment processes. The tendency to better treatment of PPCPs with longer SRTs can be seen by looking at nonylphenols and nonylphenol ethoxylate. A positive note from this table is that the lagoons which are used by a significant portion of municipalities in Canada appear to have quite good capabilities to treat organic microconstituents.

Table 1: Excerpt from CCME Terms of Reference for Project 380-2006. Estimates of removal efficiencies from TOXCHEM+ fate software (Hydromantis. Inc.)


MBR
SRT increasing

Substance	Treatment Technology						
					Tertiary		
	Facultative Lagoon	Primary Treatment	Secondary non-nitrify	Advanced Secondary	Nitrify + filter	BNR	Nitrify CAS + RO
Lead	Poor	Poor	Poor	Poor	Poor	Poor	Excellent
Lindane	Excellent	Poor	Poor	Poor	Poor	Poor	Good
Magnesium [(1)]	Poor	Poor	Poor	Poor	Poor	Poor	Good
MCPA	Moderate	Poor	Poor	Poor	Poor	Poor	Good
Mercury	Poor	Moderate	Moderate	Moderate	Moderate	Good	Excellent
Nickel	Poor	Poor	Poor	Poor	Poor	Poor	Excellent
Nitrate[(1)]	Poor	Poor	Poor	Poor	Poor	Excellent	Good
Nonylphenol	Excellent	Poor	Good	Good	Good	Excellent	Excellent
Nonylphenol ethoxylate[(1)]	Excellent	Poor[(4)]	Excellent	Excellent[(1)]	Excellent	Excellent	Excellent
pH	NA			NA	NA	NA	NA
Phenanthrene	Excellent			xcellent	Excellent	Excellent	Excellent
Phenols, Total	Excellent	Poor	Excellent	Excellent	Excellent	Excellent	Excellent
Phosphorus (total)[(1)]	Good(3)	Poor	Good	Good	Excellent	Excellent	Excellent
Pyrene	Excellent	Poor	Excellent	Excellent	Excellent	Excellent	Excellent
Quinoline	Excellent	Poor	Moderate	Good	Good	Good	Excellent

Lagoons are not practical for larger municipalities and cities because of the land area required. Treatment plants speed up and compress natural processes into a smaller foot print, minimizing land costs but increasing capital and operating costs. Membrane and other advanced technologies are generally considered cost-prohibitive at this point in time, except where water shortages are so extreme that treated wastewater is being used to replenish aquifers and even make up a portion of potable water supplies. Research is bringing costs down and improving the operating practicality of membranes.

Table 1 is an excerpt from the source table, but even the more comprehensive source table demonstrates one of the research needs in this area in that it did not include many of the substances which are currently of concern. It is also known that different substances are treated to different extents by the various treatment options. For example, there is little removal of carbamazepine even in BNR processes with longer SRTs (Chen *et al*, 2006). Galaxolide removals range from 9 – 67 % in conventional activated sludge processes (Lishman *et al*., 2006). Apparent increases are sometimes reported: Lishman *et al*., 2006 reported an increase in gemfibrozil in lagoons. More study is needed to rationalize such results.

What are the Risks?

It is uncertain what levels of risk we face from PPCPs. Many of the substances which now can be detected at trace levels have been in use for many years and have likely been in surface and drinking waters for just as many years. Firm correlations with effects have not been established, but information is increasing. Tables 2 and 3 report some typical values of endocrine-disrupting compounds (EDCs) and pharmaceutically active compounds (PhACs) in municipal wastewater effluents. Values are typically below 1 microgram/litre ($\mu g \cdot L^{-1}$).

Table 2: Typical values for Endocrine Disrupting Compounds in Municipal Wastewater Effluent, $\mu g \cdot L^{-1}$ (Chen et al, 2006; Lishman et al., 2006)

"Industrial"				
Triclosan	0.104			
Nonylphenol (NP)			1.79	ND
Bisphenol A			0.125	0.195
Bis(2-ethylhexyl)phthalate			0.394	5.2
di-n-butyl phthalate			0.137	0.246
Hormones				
17β-estradiol (E2)	ND		0.0015	ND
estrone (E1)	0.0076		0.0026	ND
17α-estradiol			ND	ND
Testosterone			ND	ND
estriol (E3)			0.004	ND
Hormone Replacements				
equilin			ND	ND
metranol			ND	ND
19-norethindrone			ND	ND
17α-ethinylestradiol			ND	0.0085
equilenin			ND	ND
Polycyclic musks				
celestolide (ADBI)	0.025	0.014-0.045		
phantolide (AHMI)	ND	0.005-0.013		
traseolide (ATII)	0.045	0.008-0.203		
galaxolide (HHCB)	0.751	0.825-1.570		
tonalide (AHTN)	0.274	0.337-0.661		
Detection limits generally in the 10's of ng/L for wastewater.				

Table 3: Typical Values for Pharmaceutically Active Compounds in Municipal Wastewater Effluent, $\mu g \cdot L^{-1}$ (Chen et al., 2006: Lishman et al., 2006)

					14 CDN cities	German WWTPs
bezafibrate		0.074	0.144	0.289		0.2
clofibric acid		ND	ND	ND	ND	ND
salicylic acid	0.106					
ibuprofen	0.384	0.284	0.383	1.15	ND	4
gemfibrozil	0.246	0.275	0.799	0.773	ND	1.3
naproxen	0.452	0.589	1.79	2.67	ND	12.5
ketoprofen	0.125	ND	ND	ND	ND	ND
diclofenac	0.194	ND	0.359	0.429	ND	ND
indomethacin	0.19	0.055	0.105	0.166		
fenofibrate						
fenoprofen		0.336	ND	0.078		ND
clofibric acid						
fluoxetine		0.509	ND	ND		
norfluoxetine		ND	ND	ND		
trimethoprim		0.106	0.907	0.795		
pentoxifylline		0.15	0.99	0.171	ND	0.5
cyclophosphamide		0.004	0.55	0.048	ND	ND
carbamazepine		0.505	0.702	1.7	0.7	2.1
caffeine		0.5	0.405	0.67		
contitine		0.029	0.165	3.48		

Detection limits generally in the 10's of ng/L for wastewater.

Table 4 presents a preliminary examination of risks focused on estrogenic activity. It compares levels of some EDCs found in the two rivers which serve as Calgary's raw water supply with results of Yeast Estrogenic Screening (YES) tests. (Note: there is a loose temporal correlation of EDC and YES results in Tables 4 and 5, but the analyses were not conducted on the same samples.) There is relatively little urban development and some non-intensive livestock farming upstream of Calgary. Low levels of estrogenic activity in these surface waters were detected in a few instances.

Table 4: Levels of Endocrine Disrupting Compounds and Estrogenic Activity in Calgary's Raw Water Supply, μg·L^{-1} (Chen et al, 2006)

EDCs in Surface Water

	Upstream Bow River	Upstream Elbow River
Nonylphenol	0.037	0.066
Bisphenol A	0.0188	0.00064
Bis(2-ethylhexyl) phthalate	1.74	0.135
Di-n-butyl phthalate	0.027	0.099
Nonylphenol ethoxylates		
NP1EO	0.025	0.020
NP2EO	0.036	0.047
NP3EO	0.039	0.032
NP4EO	0.040	0.101
NP5EO	0.042	0.238
NP6EO	0.052	0.420
NP7EO	0.061	0.676
NP8EO	0.067	0.760
NP9EO	0.060	0.841

Estrogenic Activity in Surface Water by YES* Assay

	Upstream Bow River	Upstream Elbow River
2002-10-29	<0.0014	<0.0014
2003-03-25	<0.0017	<0.0017
2003-07-15	**0.013**	<0.0017
2003-10-21	<0.0013	**0.0019**
2004-01-19	**0.0004**	**0.0090**
2004-04-27	<0.005	<0.005
2004-07-20	<0.005	<0.005
2004-10-26	<0.005	<0.005
2005-01-18	<0.0053	<0.0053
2005-04-13	<0.0027	<0.0027
2005-07-18	<0.0027	<0.0027
2005-10-18	<0.0027	<0.0027
2005-01-23	<0.0027	<0.0027
2006-05-09	<0.0027	<0.0027
2006-08-08	<0.0027	<0.0027

* YES = Yeast Estrogen Screening

Table 5 compares some levels of EDCs found in finished product water from Calgary's two drinking water treatment plants with results of YES tests conducted during the same period, although not on the same samples. Raw water for the Bearspaw Water Treatment Plant is taken from the Elbow River noted in Table 4, and the Glenmore Water Treatment Plant's raw water is taken from the Bow River. No estrogenic activity was detected.

Table 5: Levels of Endocrine Disrupting Compounds and Estrogenic Activity in Calgary's Drinking Water, μg·L^{-1} (Chen et al, 2006)

EDCs in Drinking Water

	Bearspaw	Glenmore
Nonylphenol	0.072	0.067
Bisphenol A	0.00045	0.00076
Bis(2-ethylhexyl) phthalate	0.188	0.103
Di-n-butyl phthalate	0.005	0.046
Nonylphenol ethoxylates		
NP1EO	0.030	0.024
NP2EO	0.065	0.041
NP3EO	0.043	0.025
NP4EO	0.105	0.047
NP5EO	0.122	0.083
NP6EO	0.261	0.156
NP7EO	0.414	0.223
NP8EO	0.490	0.269
NP9EO	0.551	0.297

Estrogenic Activity in Drinking Water by YES* Assay

	Bearspaw	Glenmore
2002-10-29	<0.0014	<0.0014
2003-03-25	<0.0017	<0.0017
2003-07-15	<0.0017	<0.0017
2003-10-21	<0.0013	<0.0013
2004-01-19	<0.0002	<0.0002
2004-04-27	<0.005	<0.005
2004-07-20	<0.005	<0.005
2004-10-26	<0.005	<0.005
2005-01-18	<0.0053	<0.0053
2005-04-13	<0.0027	<0.0027
2005-07-18	<0.0027	<0.0027
2005-10-18	<0.0027	<0.0027
2005-01-23	<0.0027	<0.0027
2006-05-09	<0.0027	<0.0027
2006-08-08	<0.0027	<0.0027

* YES = Yeast Estrogen Screening

The preliminary results in Tables 4 and 5 indicate no immediate health concerns for humans, but potential risks for aquatic life (Chen *et al*, 2006).

Coordination of Efforts – Other Options for Control

A national strategy for managing municipal wastewater effluents is currently being finalised by the Canadian Council of Ministers of the Environment (CCME) (information available on the CCME website). It proposes a national minimum standard of secondary treatment in Canada. This would be manifest by maximum allowable limits of 25 $mg \cdot L^{-1}$ for each of carbonaceous BOD (CBOD) and TSS. Key elements of the strategy are expected to be backed by a regulation under the Fisheries Act (information available on the Environment Canada website). These minimum requirements will also represent a certain level of removal of PPCPs. However, there are elements of the strategy which also show substantial potential for dealing with emerging issues such as PPCPs.

The development of the strategy has begun a cooperative dialogue among the three levels of government and a realization that no single approach will solve such problems.

The strategy is developing a model sewer-use by-law for source control, which is a key tool for municipalities for controlling discharges into wastewater collection systems. However, the huge number of entry points into a wastewater collection system makes monitoring and enforcement of sewer-use by-laws a significant challenge. Monitoring of ICI discharges is typically conducted by larger municipalities, but is still limited by logistics and temporal variations in discharges. Also, municipalities have little control over residential discharges, a major source of PPCPs. Residential discharges require a different approach. Bylaws can serve as guidance for residents, but are often technical out of necessity. Education could help to influence residential practices. However, municipalities have little authority over the consumer products which citizens use and discharge to the local sewer systems. This will require the involvement of the higher jurisdictions which have the potential to control consumer product formulation and perhaps even the design of substances such that they will be treated effectively by secondary wastewater treatment plants.

The CCME strategy also includes an environmental risk assessment (ERA) which expands the management of municipal wastewater far beyond the conventional parameters to include all of the substances in the CCME Environmental Quality Guidelines for surface waters. At the time of writing, it is not the intent to include the ERA in the proposed regulatory framework under the Fisheries Act except for wastewater treatment under federal jurisdiction, but provinces and territories can adopt it in their respective jurisdictions. One limitation in dealing with PPCPs is that many of these substances are not yet included in surface water quality guidelines. However, the ERA approach could easily be applied to PPCPs when surface water quality guidelines are established and the cooperative approach among the various levels of government could establish the proper control framework.

The Path Forward

There are positive signs that municipal wastewater treatment can deal with emerging issues such as PPSPs and other microconstituents. There are technical, engineering and economic challenges to scaling up new technologies and modifying the infrastructure required. Research is required both in wastewater processes, but also in the alternatives to using the problematic substances. Additional research is needed to define the environmental and health risks and to put these into the context of other risks faced in day-to-day life. Efforts to synthesize the cumulative research and an analysis of the cost benefits of the various control options are also needed. This will help to provide decision makers and the public with the information they need to decide where these issues rank in priority relative to all the other financial challenges society must balance.

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Fate and transport of drugs and personal care products following the land application of biosolids (PL)

E. Topp[1], M. Payne[2], A. Beck[3], A. Boxall[4], P. Duenk[5], D. Lapen[1], H. Li[6], C. Metcalfe[6] and S. Monteiro[4]. [1]Agriculture and Agri-Food Canada, London, ON [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON [3]Health Canada, Ottawa, ON [4]University of York, York, England [5]University of Western Ontario, London, ON [6]Trent University, Peterborough, ON

Municipal biosolids are often used as a source of nutrients for agricultural crop production. We are evaluating the fate of pharmaceuticals and personal care products (PPCPs) in soils amended with liquid municipal biosolids (LMB). Persistence and transport via either runoff or vertical movement to tile drains was investigated using commercial-scale and plot-scale field experiments conducted in Ontario Canada. Analytes of interest include gemfibrozil, acetaminophen, atenolol, ibuprofen, sulfamethoxazole, carbamazepine, cotinine, triclocarban and triclosan. All test compounds were analyzed either by LC-ESI-MS/MS or GC-MS-SIM using stable isotope surrogates for quantification, or using radioisotope methods. A variety of PPCPs were detected at ng·L^{-1} to mg·L^{-1} concentrations in both tile drainage and surface runoff. The concentrations of PPCPs in surface runoff or tile drainage from plots receiving subsurface injected biosolids were much lower than from plots receiving a broadcast application followed by incorporation. Selected analytes (e.g. carbamazepine) were detected in runoff water at least 9 months post-application. Overall, these data indicate that there is potential for transport of small amounts of PPCPs from agricultural land that has received biosolids, and that this risk can be managed by appropriate biosolids application practices. Results will be discussed in the context of published toxicological endpoints.

Neurotoxicological effects of a primary and ozonated treated wastewater on freshwater mussels exposed to a real-time flow-through system (PL)

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The neurotoxic potential of a primary-treated and ozonated municipal effluent was examined using feral freshwater *Elliptio complanata* mussels. Specimens were exposed to increasing concentrations (0, 1, 3, 10 and 20 % v/v) of a primary-treated effluent before and after treatment with 10 mg·L^{-1} of ozone in a mesocosm-type experiment for seven weeks. A suite of biomarkers was used to assess the potential neurotoxic stress of the wastewaters on these benthic invertebrates: opiate binding sites, γ-aminobutyric acid (GABA), metabolism, monoamine (serotonin, dopamine, monoamine oxidase), and acetylcholinesterase and lipid peroxidation. Gamete activity was also determined by the gonado-somatic index and by vitellogenin-like proteins. The results show that the number of opiate binding sites increased slightly, especially after ozonation. GABA metabolism was generally reduced, suggesting higher glutamate stimulation than GABA dampening effects in mussel ganglia. This excitatory state was further confirmed by decreased acetylcholinesterase activity in gonadal tissues. The turnover of dopamine was enhanced with increased serotonin levels, but accompanied by reduced catabolism, as evidenced by decreased monoamine oxidase activity. Moreover, oxidative stress was increased, as determined by lipid peroxidation in the gonad (containing ganglia), which was significantly correlated with acetylcholinesterase activity and dopamine metabolism. The gonado-somatic index was significantly reduced with increased levels of vitellogenin-like proteins, again confirming the estrogenic action of these wastewaters. The data suggest that exposure to a primary-treated municipal effluent before and after ozonation leads to an excitotoxic syndrome implicating perturbations in GABA, dopamine and acetylcholine signaling. The increase in dopamine metabolism may be associated with the occurrence of opiate-like compounds (i.e. morphine) in the effluent. In general, ozonation reduced the severity of the responses, indicating that this disinfection strategy does not increase neurotoxicity to mussels.

Effects of municipal wastewater effluent on reproductive function in wild fish exposed in a small receiving environment (PL)

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Studies by Environment Canada on Wascana Creek in Regina, Saskatchewan, Canada, have determined that during the winter low flow periods the creek is 100 % treated sewage making it a model system to understand the effects of MWWE. In this study, we were interested in determining whether MWWE effects reproductive development in fish in Canada, and if so, does the impairment influence the survival of fish populations. In 2007 and 2008, field studies assessed sentinel fish species under pre-spawning, spawning and post-spawn conditions in terms of growth (condition factor), and reproduction (in vitro sex steroid capacity, vitellogenin, gonadosomatic indices, and histology). Comparisons were made in fish collected upstream and downstream of Regina's Wastewater Treatment Plant. Abundant species in this system are the fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*). Both sexes of spawning fathead minnow collected downstream of the sewage discharge were smaller, had reduced condition and larger liver somatic indices when compared to fish collected upstream of Regina. MWWE exposed males also had elevated levels of vitellogenin and lower scores of secondary sexual characteristics (fewer nuptial tubercles, little or no development of the dorsal pad, and no dorsal fin dot). Exposed female stickleback had increased gonad size and corresponding increased testosterone production but decreased 17B-estradiol productive capacity compared to reference fish. Post-spawning collections revealed an absence of sufficient mature fathead minnows downstream of the MMWE discharge for site comparisons, although stickleback were abundant and did not demonstrate any significant site differences in performance.

The anti-depressant venlafaxine: distribution in surface water and effects on the feeding of Japanese Medaka (*Oryzias latipes*) (PL)

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Venlafaxine is one of several drugs in a class of anti-depressants called Serotonin Norepinephrine Re-Uptake Inhibitors (SNRIs). We evaluated the distribution of 6 antidepressants (venlafaxine, bupropion, fluoxetine, sertraline, citalopram and paroxetine) and 5 of their metabolites in municipal wastewater and in surface water. Of these compounds, venlafaxine and its active metabolites, O- and N-desmethylvenlafaxine were found at the highest concentrations ($>0.5\ \mu g \cdot L^{-1}$) in sewage effluents and in surface water downstream of municipal wastewater treatment plants in the Grand River watershed in Ontario. To understand the potential impacts of anti-depressants on fish exposed to these compounds in urbanized watersheds, we evaluated the influence of venlafaxine on food intake in the Japanese medaka (*Oryzias latipes*). In our experiment, fish were exposed to venlafaxine at nominal concentrations of 3.2, 32 and 320 $\mu g \cdot L^{-1}$, with one control treatment. Food uptake was measured using two methods, a clearance model and a tracer method using food spiked with enriched ^{110}Cd isotope. The results from two trials with the clearance model indicate that there were no effects on food consumption in medaka exposed to 3.2 and 32 $\mu g \cdot L^{-1}$ venlafaxine. In the highest treatment (i.e. 320 $\mu g \cdot L^{-1}$), the medaka consumed significantly less food than fish from the control treatment. Data are currently being analyzed to determine whether the tracer method shows similar trends. Data will be presented on the concentrations of venlafaxine and its active metabolites in the tissues of exposed medaka.

An ecosystem study of presence and effects of pharmaceuticals and nutrients in the aquatic environment: Wascana Creek, SK (PL)

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Worldwide, pharmaceuticals are increasingly detected in surface waters receiving treated sewage effluent. The city of Regina, Saskatchewan, (population 190,400) treats its sewage at a modern tertiary sewage treatment plant (STP) on Wascana Creek. Four water surveys conducted from winter 2005 to spring 2007 indicated that mixtures of pharmaceuticals were always present downstream of the STP in $ng \cdot L^{-1}$ and sometimes $\mu g \cdot L^{-1}$ concentrations. This mixture included antibiotics, analgesics, anti-inflammatories, lipid regulators, caffeine and nicotine metabolites and DEET. Nitrogen and phosphorus concentrations were much higher downstream compared to an upstream site. In fact, un-ionized ammonia concentrations far exceeded concentrations in the Canadian Water Quality Guidelines for the Protection of Aquatic Life. Of concern is the fact that creek biota is chronically exposed to low concentrations of pharmaceuticals and high concentrations of nutrients over long time periods. Although the risk is currently unknown, this study suggests that ecosystem level changes may already be occurring. High ammonia concentrations from the STP may be responsible for depressions in algal biomass and production observed. As well, changes in the primary to bacterial production ratio, and a disconnect between total phosphorus and chlorophyll *a* and bacterial production and Chl *a*, may be indications that pharmaceutical and nutrient exposure presents a risk to aquatic organisms in the creek.

Effects of triclosan exposure on the development of river biofilm communities (PL)

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Triclosan (TCS) is a common-use antibacterial agent found in a wide range of personal care and consumer products. However, there is a dearth of information on its' effects in the environment. River biofilms were exposed to TCS continuously at $10\ mg \cdot L^{-1}$ or for varying periods at $2\ mg \cdot L^{-1}$. Microscale analyses indicated a significant increase in bacterial biomass at $10\ mg \cdot L^{-1}$ triclosan, accompanied by significant reductions in algal and cyanobacterial biomass ($p < 0.05$). Molecular analyses confirmed significant changes in bacterial community composition. When biofilms were allowed to develop prior to exposure or exposed to TCS and then allowed to recover biofilms which were cultivated in the absence of TCS and then subsequently exposed all exhibited reductions in algal biomass and cyanobacterial biomass. Pigment analyses indicated changes in autotrophic community composition. Bacterial biomass was not affected. Unique community endpoints were observed for all treatments. Protozoan populations were suppressed by TCS at 2 mg·L^{-1}. Community structure relative to control communities was altered by any exposure to TCS. The results indicate that even relatively brief exposure of a river biofilm community to low levels of TCS will alter both the trajectory and endpoint of development.

The effects of fluoxetine, ibuprofen, and CTAB on the photosynthetic activity of *Lemna minor* (PO)

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Pharmaceuticals and personal care products have been found in surface waters in the environment, having been released from sewage treatment plants. The present study was conducted to assess the effects of two pharmaceuticals, Fluoxetine and Ibuprofen, and a surfactant, CTAB, on the photosynthetic activity of the aquatic plant *Lemna minor*. *L. minor* fronds were exposed to either 1 mg·L^{-1} Fluoxetine, 1 mg·L^{-1} Ibuprofen, 0.1 mg·L^{-1} CTAB or appropriate solvent controls for 7 days, with exposures replaced every other day. Photosynthetic activity was assessed by measuring oxygen production from photosystem II (PSII). Chlorophyll content and growth also were determined. Exposure to Fluoxetine, Ibuprofen, and CTAB caused a reduction in oxygen production with exposed fronds having PSII activities that were 76.2±14.7 %, 76.0±23.3 %, and 73.6±9.5 %, of their solvent controls respectively. Exposure to Fluoxetine significantly increased total chlorophyll content while Ibuprofen and CTAB had no significant effect. Exposure to Fluoxetine resulted in a significant increase in chlorophyll a content but had no significant effect on chlorophyll b content. Exposure to Ibuprofen and CTAB had no significant effect on either chlorophyll a or b content. Exposure to the three compounds had no significant effect on growth. Although the concentrations of pharmaceuticals used in this study are higher than those found in the environment, these results indicate that higher levels of exposure to Fluoxetine, Ibuprofen, and CTAB, can reduce the photosynthetic activity of *L. minor*.

The effects of pharmaceutically active compounds on the freshwater oligochaete, *Lumbriculus variegatus* (PO)

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Pharmaceutically active compounds (PhACs) are entering freshwater systems through sewage treatment plants via human waste. The effects of PhACs on aquatic wildlife are unknown. The freshwater oligochaete, *Lumbriculus variegatus* was exposed to three PhACs (Ibuprofen (IBU): 10 mg·L^{-1}, Fluoxetine (FLU): 1 mg·L^{-1}, and Cetyltrimethylammonium bromide (CTAB): 0.1 mg·L^{-1}) or their respective solvent controls to monitor behavior, growth, and O_2 consumption. Changes in body reversal and helical swimming behavior were monitored on days 0, 2, and 6 post-exposure. Regeneration of segments was measured on days 0 and 6 after exposure of ablated worms. O_2 consumption assays were run on day 2. On day 2, IBU and FLU reduced body reversal behavior to 45.6 % ± 30.8 % and 91.3 % ± 15.5 % of the solvent controls respectively and helical swimming to 20.8 % ± 29.7 % and 84 % ± 21 % with further reductions in behavior on day 6. CTAB did not significantly alter behavior. Although not statistically significant, FLU and CTAB decreased growth while IBU increased growth in comparison to their respective solvent controls. No significant differences were observed in the mean O_2 consumption for IBU or FLU. However, measurable O_2 consumption was delayed significantly in IBU exposed worms. CTAB significantly reduced O_2 consumption to 68.8 % ± 11.9 % of the solvent controls. Results indicate that IBU and FLU have a significant effect on *L. variegatus* behavior with helical swimming the more sensitive behavior. Behavior appears to be a more sensitive biomarker than growth or O_2 consumption.

Contaminants analysis in effluents from the three municipal wastewater treatment plants from the city of Laval (QC): preliminary study (PO)

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In recent years, concern has been growing over municipal wastewater effluents and the fact that they represent a source of pharmaceutical and personal care products for the receiving waters. The goal of this preliminary study was to make an exhaustive chemical characterization of the discharges of the wastewater treatment plants of the city of Laval. This study completes an ongoing ecotoxicological analysis on those discharges. Discharges from Auteuil, La Pinière, and Fabreville stations were sampled two times during winter 2007. Approximately 330 chemical parameters were analysed in these samples: steroid hormones, diverse pharmaceutical products, pesticides, perfluoro-compounds, and other categories of contaminants. Even though the measured concentrations seem relatively weak, uncertainty persists about the potential effects on receiving waters since the toxicity of several of these substances is, at best, poorly documented. Also, we observe important inter- and intra-stations variability, both qualitatively and quantitatively. In this presentation, the emphasis is mainly put on the presence of emerging chemicals of concern and on the identification of parameters which can serve as tracers for follow-up studies. Financed by the city of Laval, Environment Canada, and CIRÉ.

Effects of anti-microsporidial agents on fish cell lines (PO)

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Microsporidia are spore-forming, obligate intracellular parasites known to affect almost every group of animals including humans. Species of microsporidia infecting humans have been identified in water sources as well as in wild, domestic, and food-producing farm animals, raising concerns for waterborne, foodborne, and zoonotic transmission. Recent reports of *Heteroporis* sp. infections in fish within the Great Lakes have caused major concerns and prompted the inclusion of these microsporidian species as one of 25 Priority Invasive Species by the Great Lakes Panel on Aquatic Nuisance Species. A watch for these emerging pathogens is in effect, and no effective treatments have been reported. Current therapies for microsporidiosis include Albendazole which is a benzimidazole that inhibits microtubule assembly and is effective against several microsporidia. Fumagillin, an antibiotic and anti-angiogenic compound produced by *Aspergillus fumigatus*, is more broadly effective but is toxic when administered systemically to mammals. The effectiveness or toxicity of these compounds have not been evaluated in fish thus, several fish cell lines including an epithelial cell line from the Japanese eel (EP-1), persistently infected with *Heterosporis anguillarum* were evaluated for drug efficacy and safety. Fumagillin at concentrations from 0.1 to 100 $\mu g \cdot mL^{-1}$ were evaluated in non-infected fish cells as well as in EP-1. Chemotherapeutic treatment of EP-1 with Fumagillin as well as with Albendazole, Norfloxacin, Ofloxacin, and Amphotericin B decreased the number of spores in a dose dependent manner but none completely cleared infection with a range of concentrations from 1 to 15 $\mu g \cdot mL^{-1}$.

Investigation of community, population and individual responses of fish exposed to multiple municipal wastewater effluents in Canada (PO)

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Municipal Wastewater Effluent discharge (MWWE) is the largest by volume discharge into the Canadian aquatic receiving environment and discharge rates will rise with projected increased human population density in urban areas. MWWE or sewage is a mixture of domestic and industrial wastes, and Pharmaceuticals and Personal Care Products (PPCPs). The Grand River watershed in the province of Ontario receives the outflow of 26 sewage treatment plants and runoff from agriculture, aggregate and industrial activities. In 2005 and 2007, field studies assessed fish communities (diversity and abundance), populations (age-distribution, growth rates) and individual responses in terms of growth (condition factor) and reproduction (in vitro sex steroid production, gonadosomatic indices, histology). Comparisons were made in fish collected upstream and downstream of 2 municipal wastewater treatment plants where existing National Water Research Institute (NWRI) studies have measured detectable levels of a number of pharmaceuticals. Fish [greenside darter (*Etheostoma blennioides*) and rainbow darters (*Etheostoma caeruleum*)] collected downstream of both plants were longer and heavier when compared to reference fish collections, and demonstrated increased condition downstream of the Kitchener and Waterloo municipal wastewater plants. This could be a reflection of the increase diversity and abundance of the benthic invertebrate community observed downstream of the discharges. Although fish populations did not display effects to MMWE exposure, individual exposed fish demonstrated physiological alterations in sex steroid productive capacity. Fish community assessments also demonstrated increases in fish abundance, diversity and evenness in the composition of the river fauna downstream of the MMWE discharges when compared to reference fish communities with similar habitat characteristics.

NOVEL BIOLOGICAL TEST METHODS

Protecting Canada's drinking water: developing real-time, early-warning biomonitoring technology (PL)

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Our research addresses the necessity of developing an early-warning strategy to detect potential threats to drinking water sources. Natural bodies of water that ultimately constitute some of Canada's drinking water supply can contain a variety of pollutants such as heavy metals, PAHs, halogenated organic molecules, pharmaceuticals, and pathogens. While the associated health risks of some of these components are already known, there remains an alarming lack of research in many other areas. We are developing a holistic, multi-organism-based system to measure stress reactions in the aquatic community and are monitoring these stress responses in real-time. We will also develop ecotoxicity models based on dose-responses of individual organisms to specific classes of stressors (chemical and biological). These models will aid in the interpretation of the data and will provide information for water-utilities managers about the nature of the stressor. The ultimate goal of our research is the development and implementation of an early-warning system in real-time for drinking-water facilities that would detect chemical contaminants and pathogens using biomonitoring organisms. Our study is/will: 1) measuring responses in aquatic plants and invertebrates to chemical stressors at environmentally-relevant concentrations, and to pathogens; 2) developing a microarray-based test that can be used to directly detect pathogenic organisms; 3) build and test a flow-through system in a drinking-water facility for stress-response determinations in real-time; and 4) develop methods of stereotyped responses for the whole suite of biomonitoring organisms to chemical contaminants and pathogens.

The use of freshwater aquatic invertebrates in a real-time, early-warning monitoring system designed to protect Canada's drinking water resources (PL)

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Recent anthropogenically-induced changes to the environment have raised concerns about the quality of drinking water in North America. One emerging method of monitoring water quality is the use of Biological Early -Warning Systems (BEWS), systems which employ living organisms to assess potential impacts of contaminants in drinking water sources. By monitoring the changes in behaviour and physiology of test organisms, it is possible to detect impacts from sub-lethal concentrations of contaminants which cause stress to organisms and may be harmful to human health. This research focuses on four invertebrate organisms, including the water column zooplankter *Daphnia magna*, the benthic amphipod *Hyalella azteca*, the aquatic worm *Lumbriculus variegatus* and the larval insect *Chironomus tentans*, and one vertebrate species, the larval amphibian *Rana catesbeiana*. The effects on various behaviours in response to sub-lethal concentrations of Atrazine, a commonly used agricultural pesticide, were evaluated. Using still photography and digital video analysis, changes in swimming behaviour of *D. magna*, *H. azteca*, *C. tentans*, *R. catesbeiana* and *L. variegatus* colonies were examined. Digital video analysis was also used to monitor changes in phototactic response in *D. magna*, pre-copulatory mating behaviour in *H. azteca* and avoidance behaviour in *C. tentans*. Stress was also measured by examining respiratory behaviour of *C. tentans* colonies and the *R. catesbeiana* larvae using dissolved oxygen probes. Future applications of this research will lead to the development of a continuous monitoring system using behavioural changes of living organisms which will prevent contaminants from reaching public drinking water supplies.

Development of a biological, early-warning system (BEWS) in real-time using automatic image analysis and biochemical probes to rapidly detect potentially deleterious freshwater environmental conditions (PL)

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Freshwater sources for residential, commercial and industrial uses are in high demand and also exist in limited quantities. Developing a biological early-warning system (BEWS) to rapidly assess aquatic toxicity is important to reduce human health risks. Our model includes a number of different organisms which demonstrate a variety of responses to stressors in aquatic environments. A selection of flora and fauna encompasses a large range of toxicity endpoints. *Lemna gibba* or duckweed is a free-floating macrophyte which can be monitored using video analysis for differentiated growth rates in the presence of stressors. Photosynthetic output of the green algae, *Pseudokirschneriella subcapitata*, is monitored using dissolved oxygen and pH probes to rapidly identify aquatic conditions. Using digital cameras, valve behaviours of freshwater mussels, *Pyganodon grandis*, are monitored for short-term physiological responses while the stress behaviours of *Hydra viridissima* are similarly monitored by video analysis. Under certain adverse conditions, *Hydra* will change from its normal elongated state into a retracted form. *Euglena gracilis* is a microscopic organism having both animal and plant-like components. Its phototactic and gravitactic behaviours allow for sub-acute identification of contaminants using image analysis software. Tributyltin, an antifouling paint used on boats, is evaluated in increasing concentrations to identify behavioural changes of these organisms. The use of environmentally-relevant organisms from different trophic levels of the aquatic food web is ideal to determine toxicity in aquatic environments.

Defensible selenium tissue residue guidelines based on larval fish deformities (PL)

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Selenium (Se) tissue residue guidelines (TRGs) from fish reproductive toxicity testing are based on measurement of larval deformities using a graduated severity index (GSI) approach. These GSI data comprise one-half of the residue-response relationship used to derive a TRG. Explicit evaluation of all sources of uncertainty associated with a TRG is critical, given its importance in both hazard and risk assessments; however, this evaluation is currently limited to items such as tissue chemistry data or the statistical methods used to describe the residue-response relationship. Evaluation of the quality of the GSI data (which forms the response axis) has generally been absent. An incomplete uncertainty assessment is problematic because it contributes to a false sense of confidence regarding the accuracy and precision of the TRG. This presentation summarizes the necessary quality assurance/quality control (QA/QC) measures that must be incorporated into the larval deformity assessment component of Se reproductive toxicity testing, including: establishing clear a priori descriptions for each GSI rating; dealing with preservative-induced changes to edema and other deformities; reducing the influence of observer drift during the assessment; providing appropriate external verification of GSI scores; and reporting requirements. The potential influence of uncertainty on GSI data and implications for the final TRG are illustrated with example data from the literature.

ECOLOGICAL RISK ASSESSMENT

The use of derived effluent release limits to guide the environmental design of a new uranium mine (PL)

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The Millennium project is a potential uranium mine located in northern Saskatchewan, Canada that has yet to enter the formal environmental assessment process. Project approval under the *Canadian Environmental Assessment Act's* screening and comprehensive environmental assessment processes is contingent on showing that the project will not likely cause significant adverse effects. Although this determination is dependent on many factors, it is generally accepted that if the predicted concentrations in the environment and/or doses to Valued Ecosystem Components (VECs) are less than toxicity reference values (TRVs), significant adverse effects are not expected. As a result, one of the environmental design objectives for the Millennium project was to have concentrations of constituents of potential concern (COPCs) in the receiving environment less than TRVs. One of the highest profile environmental impacts from mining is the long-term release of treated mine effluent into the aquatic environment. As such, effluent design (quantity, quality and point of discharge) is critical to ensure significant adverse effects are avoided. The ecological risk model employed can determine the assimilative capacity of the receiving environment and back-calculate derived effluent release limits (DERLs) based on the most sensitive aquatic receptor. Because the Millennium project is still in the pre-feasibility stage, this type of environmental screening analysis was used to determine whether or not further mitigation and/or treatment is required. Moon Lake (the proposed receiving environment) was considered in four configurations to calculate DERLs and determine the optimal point of discharge. Comparisons revealed that discharging into the Moon Lake's northwest basin (the original design) would exceed more TRVs for selected COPCs than discharging into the main body of Moon Lake. Based on these results and the fact that the predicted effluent quality is based on best available practicable technology, relocation of the point of discharge is currently planned. Future work is under way to move beyond the screening-level assessment by refining the inputs and assumptions of the model. This includes assessing upper bound treated mine effluent and contingency releases and compartmentalizing the main body of Moon Lake to get a more accurate representation of near field and far field effects.

Ecological and human health sediment risk assessment for a hydrocarbon-impacted site in Lake Athabasca (PL)

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Historical operation of a public port facility near Uranium City, Saskatchewan, contributed to elevated concentrations of hydrocarbons in soil, groundwater and sediment. The uplands portion of the site was successfully remediated; however, a risk management approach was implemented to resolve human health and ecological issues related to the aquatic portion of the site. Ecological risks were assessed using a sediment weight-of-evidence approach consisting of chemistry, toxicity, bioaccumulation and benthic community structure, while human health risks via fish consumption, water ingestion and direct contact were evaluated following Health Canada guidance. This presentation provides an overview of the general risk assessment approach and site-specific data and findings, but focuses primarily on the challenges encountered during the risk assessment process. Among the key challenges was the need to include alkylated PAHs as a COPC in the human health risk assessment and to evaluate ongoing propeller wash and sediment resuspension as a factor influencing sediment risk management options, despite closure of the port facility.

A synopsis of long-term aquatic effects monitoring after an oil spill to Wabamun Lake, Alberta (PL)

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A CN train derailment during the early hours of August 3, 2005, resulted in the release of Bunker 'C' Oil into the waters of Wabamun Lake. In response to the spill, Alberta Environment issued an *Environmental Protection Order* requiring CN to prepare a Long-term Monitoring Plan, one objective of which was to monitor the effects of the spilled oil on the aquatic environment. In the three years since the spill, monitoring has included assessments of potential effects on plankton, benthic invertebrate, emergent vegetation, and fish communities. In the process, we collected ~2,000 sediment grabs for chemistry, toxicity, and benthic invertebrate analysis, ~700 L of water for chemistry and toxicity analyses (and filtered a further ~1,200 L of water per sampling event for plankton taxonomy) and ~40 kg of emergent vegetation for measuring biomass. Analytical techniques consisted of a variety of laboratory and field experiments, including the deployment of almost 200 semi-permeable membrane devices to measure exposure to hydrocarbons, as well as 4,000 whitefish and 12,000 northern pike eggs for an *in situ* assessment of larval fish development. Finally, 38 lines of evidence from the initial, intensive studies were integrated into one weight of evidence framework, and follow-up studies were conducted where potential effects related to the spilled oil were identified. As a result of this extensive set of studies, we concluded that the oil spill did not cause wide-scale effects in Wabamun Lake.

Beyond the sediment quality triad: Trent River sediment dioxin-furan investigation (PL)

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The sediment quality triad approach, which includes sediment chemistry, sediment toxicity, and benthic community alteration, is not always enough to determine whether sediment management actions are needed at some sites. Application of the Canada-Ontario Decision-Making Framework for Assessment of Contaminated Sediment, which includes a fourth line of evidence, the potential for contaminant biomagnification, was applied to data collected from the lower Trent River, Trenton, Ontario. Results indicated that there was a need to fully assess the risk of biomagnification due to elevated concentrations of dioxins and furans and dioxin-like PCBs in mayfly, caged mussel, and young-of-the-year yellow perch tissues. Consequently, a detailed Ecological Risk Assessment (ERA) was conducted. Lower chlorinated congeners were accumulated in tissue of young and adult fish, but there was little accumulation of the higher chlorinated congeners in biota despite high concentrations of these congeners in sediment and benthos. Although there was potential risk to some sensitive fish species through consumption of contaminated food, risks to receptor species (mallards, mergansers, great blue herons, double-crested cormorants, and mink) were estimated to be negligible based on conservative assumptions of the exposure of these species to the chemicals of concern. The ERA concluded that remediation of the sediment deposits did not appear to be warranted at this time on the basis of predicted risks to biota.

Common pitfalls in weight of evidence sediment quality assessments (PL)

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The Sediment Quality Triad (SQT) and other weight of evidence (WOE) approaches provide frameworks for integrating multiple lines of evidence (LOE) in sediment quality assessments. Each LOE contributes different information and different uncertainties. Challenges with WOE implementation arise from conflicts between prescriptive, quantitative, and standardized approaches at one extreme, and adaptive approaches incorporating best professional judgment at the other extreme. Dubious procedures that should be avoided include: disregarding the benthos in sediment quality assessments; pursuit of a single "best" toxicity test / species for all conditions; decision rules that reflect only statistical significance or effect size; lack of consideration of substrate characteristics and ancillary parameters; excessive reliance on bulk sediment chemistry; equating correlation with causation; assuming that longer toxicity tests are necessarily better tests. These and other practices undermine the goals of sediment quality assessments. Case studies demonstrate the relevance of these issues to SQT / WOE development and guidance. Recommendations are made for adjusting SQT / WOE designs to explicitly address these issues and minimize the resulting uncertainties. For example, in the specification of decision rules for evaluating effects, all four elements of statistical significance need to be considered (sample size, effect size, significance level, data variance). Solutions do not require application of complex techniques or excessive resources; they only require acceptance of the importance of site-specific customization of WOE assessments (to increase ecological relevance) and clear documentation of processes and assumptions (to keep decisions transparent).

Big projects, past legacies, and planning the future with a harmonized view: Environmental perspectives (PL)

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The complement of short and longer-term activities produces a footprint on the environment. The magnitude of that footprint relative to those of naturally-occurring and other anthropogenic factors must be accounted for in order to gain perspectives on potential impacts on the environment relative to baseline conditions that can be linked to other, unrelated processes or activities. For example, when planning large projects such as mining, reactor new-build or long-term waste disposal, or when evaluating the need to mitigate existing or legacy situations related to past or current activities, it becomes necessary to plan and carry out activities with a balanced view that weighs out the potential costs versus the benefits of those activities. Such considerations must be taken in the context of the laws which govern environmental protection and the sustainability of natural resources for current and future generations. In addition, it is necessary to engage key stakeholders, including the public and regulatory agencies amongst others, to provide a forum in which it is possible to define a consensus-based and accountable path forward that is in line with regulatory requirements while addressing potential issues of broader interest, such as the availability of safe, reliable and responsible energy sources. Integral to such interactions are the project-specific drivers underlying them in the community or country where the activity is being planned. These concepts will be presented in the context of perspectives gained over the past several years as an industry expert and Canadian representative on both national and international committees focused on environmental protection, stewardship and compliance in the nuclear industry.

Developing biological indicators of ecosystem health for south and central Saskatchewan (PL)

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A monitoring program was initiated to provide biological measures of ecosystem health for incorporation into the State of the Watershed reporting by the Saskatchewan Watershed Authority. In 2006 and 2007, benthic macroinvertebrate and related watershed data were collected for approximately 200 sites across south and central Saskatchewan. Data collected from sites least impacted by human activities were used to model the relationships between biological and habitat attributes in the region. These empirical models provided site-specific biological benchmarks that were used to assess the biological condition of sites potentially impaired by human activities. Assessments of biological condition were derived by comparing the site-specific benchmark to the actual biological condition of each site using the Test Site Analysis method (TSA). TSA provides the probability a given site has been impaired by human activity and information on how it has been impaired. This empirical approach to bioassessment provides an ecologically meaningful and statistically rigorous summary of biological health that can be readily interpreted and used to improve decision making and watershed management.

Understanding sculpin recovery following a NaOH spill into the Cheakamus River, BC (PL)

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In 2005, the Cheakamus River sculpin populations were affected by an accidental discharge of sodium hydroxide. Although the product did not persist, it caused an estimated 98 % reduction in sculpin densities. Adding to this impact is the limited knowledge concerning cottids in the system. The Cheakamus Ecosystem Restoration Technical Committee--comprised of regulatory agencies, local government, the Squamish Nation and CN--was formed to evaluate the impacts of the spill, as well as to promote the subsequent recovery of fish populations in the river. The Committee opted for a natural recovery strategy for sculpins, with a multi-year monitoring plan to assess sculpin abundance and recovery. Through adaptive management, the 2007 monitoring plan was revisited to include considerations such as spatial distribution, population structure and genetic diversity, as they too are important components of recovery. Projects include monitoring of sculpin migration and investigations of population distinctiveness among neighbouring systems, as well as sampling various life stages. This work has promoted the collaboration of multiple agencies and stakeholders, fostered educational opportunities at the community level, and led to research employing microsatellite DNA analyses, and a research project for a M.Sc. candidate. Results of the monitoring program are providing insight into the response and recovery of sculpins to pulse contamination and the ecology of the species in the study area.

Toxicity of perfluorooctane sulfonate (PFOS) to avian wildlife: ambient safe water value derivation and uncertainty analysis (PL)

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Possible risks of exposure of avian wildlife to perfluorooctane sulfonate (PFOS) were evaluated. Toxicity reference values (TRVs) and protective water values (WVs) were derived using existing toxicological data. These benchmark values provide a point of reference for acceptable concentrations of PFOS in the environment and, in particular, to avian species. They represent threshold values in food and tissues of a trophic level-4 avian predator that are protective of other species that may be exposed to PFOS through aquatic food chains. The benchmarks were based on acute and chronic exposures of Northern bobwhite quail and mallard that were exposed via their diet. Toxicological endpoints included mortality, growth, feed consumption, and histopathology. Reproductive endpoints included egg production, fertility, hatchability, and survival and growth of offspring. Uncertainty factors that accounted for species extrapolation, exposure duration, and toxicological relevance were derived using the Great Lakes Initiative (GLI) methodology. Avian Wildlife Values (WV) were derived using bioaccumulation and biomagnification factors to extrapolate threshold tissue PFOS concentration to water concentration for three avian species: the bald eagle, kingfisher, and herring gull. Based on these analyses, species-specific safe water concentrations for bald eagle, kingfisher, and herring gulls were 71 $ng \cdot L^{-1}$, 36 $ng \cdot L^{-1}$, and 41 $ng \cdot L^{-1}$, respectively. The overall avian wildlife value was determined to be 47 ng PFOS·L^{-1}. To better understand the uncertainty associated with these values relative to their environmental relevance, a sensitivity analysis was conducted that evaluated bioaccumulation and dietary assumptions of the extrapolation model. The results from these analyses are discussed relative to current freshwater PFOS concentrations measured in Great Lakes waters.

Development of no-effect values for metals in sediment for use at uranium operations in Northern Saskatchewan, Canada (PO)

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No-effect values for metals in sediment were derived and compared to sediment quality guidelines that are currently in use in northern Saskatchewan. This investigation was motivated by the hypothesis that metal concentrations in sediment found at field sites where there is no measurable effect on the benthic community may be considered "safe" at the site-specific level. In order to examine this hypothesis, no-effect values for metals in sediment were derived using data from uranium operations in northern Saskatchewan, Canada, as a case-study. To calculate no-effect values, paired data sets of benthic community metrics and bulk sediment total metal concentrations were obtained from current and historical reports from uranium operations. Benthic communities at reference and potential exposure sites were deemed to be not adversely affected if they were not statistically different from the reference site in either abundance or richness. Bulk sediment concentrations of As, Cr, Cu, Pb, Mo, Ni, Se, U, and V at no-effect sites were each plotted as a cumulative frequency distribution. A logistic model was used to fit a curve to the data. The no-effect value for each metal was interpolated from the line of best fit at the 90th centile. No-effect values were then compared with existing sediment quality guidelines for the metals of interest. A summary of the results and comparisons is discussed. The findings from this assessment have implications for the development of updated sediment quality guidelines.

Sensitivity of early white sturgeon (*Acipenser transmontana)* life-stages to copper, cadmium, and zinc (PO)

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Poor recruitment of white sturgeon (*Acipenser transmontana*) in the Columbia River has been documented since the 1970s. There are many possible causes for this phenomenon, including changes in habitat, nutrition, predation and genetics. One of the hypotheses that has been put forward is that exposure to metals may influence survival of eggs and or juveniles. In general, little is known about the potential toxicity of metals such as Cu, Cd, and Zn to white sturgeon. The purpose of this study was to establish baseline laboratory toxicity data on the exposure of early life-stages of white sturgeon to Cu, Cd, and Zn which can be used in risk assessments and, in combination with field experiments conducted in a parallel study, to assess the potential toxicity of these metals in waters of the Columbia River. Laboratory studies were conducted using a flow-through exposure system adapted for controlled experiments of white sturgeon. Embryos, larvae, and fry were exposed to increasing concentrations of dissolved Cu, Cd, and Zn for 60 days. Endpoints included hatchability, mortality, growth, and development of larvae. In addition, 96hr LC50 static toxicity tests were conducted for each metal in order to gather information to calculate water effect ratios (WER) between laboratory and separate concurrent field studies. The species-specific dose-response relationships will be used to establish metal toxicity threshold values for white sturgeon exposed to Cu, Cd, and Zn. This information will be used along with metal speciation models to predict thresholds for effects of these metals on early life-stages of white sturgeon under field conditions.

Weight-of-evidence approach to assess environmental impact (PO)

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In this work, we used a weight-of-evidence approach to assess potential impacts to the Swan River from the associated facilities. The Swan River runs east to west across the north-central part of the Swan Hills Field. The Swan Hills Field consists of 387 operating, suspended, or abandoned oil and gas well sites. Four downstream stations and one upstream (reference) station were included in the study. The weight-of-evidence approach used incorporated both the degree of response for each identified endpoint (a measurable biological variable or chemical concentration) and the degree of agreement amongst endpoints at each of the sampling stations. Potential effects on aquatic indicators related to a reduced environmental quality were identified. All endpoints included were given equal weight. Endpoints assessed included: water chemistry data; sediment chemistry data; sediment toxicity data; and benthic community data. All stations received rating scores of "moderate potential effects" for water chemistry and sediment chemistry. Using sediment toxicity, no downstream sampling locations were statistically different ($p = 0.05$) from the upstream reference station for any parameter, and therefore all stations received rating scores of "negligible/low potential effects" for sediment toxicity. No significant differences between the reference and downstream sampling locations were noted for any the community metrics. Also, pollution-sensitive taxa (e.g., EPT) are the dominant taxa and pollution tolerant taxa (e.g., oligochaetes) are rare. Therefore, with respect to the score as per the weight-of-evidence approach, the benthic invertebrate ratings were negligible/low.

Predictive ability of sediment quality guidelines and design of a Tier 1 risk assessment framework for dredged sediments: how to deal with confounding factors in practice? (PO)

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Many tiered frameworks designed for contaminated sediment risk assessment rely upon sediment quality guidelines (SQG) at the first tier. In case of multiple contaminations, results can be aggregated in indices such as mean quotients. It can thus be decided, for example, to dispose of dredged materials in open water without further investigation, provided SQGs or specific values of indices derived from SQGs are not exceeded. Thus, the relevance of SQGs, and indices as well, is critical for environment protection. In the context of the development of a tiered framework for dredged materials assessment of the St Lawrence River, we assessed various indices based on the SQGs available for this stream and a database matching chemistry and toxicity tests. As the overall efficiency of any of the tested indices remained rather low, factors such as sediment grain size, nutrients, and metal-binding phases, which could explain type II errors (false negatives), were examined. This led to the design of a modified Tier I, where SQGs are used in combination with decision-rules based on some explanatory factors. This work is supported by Environment Canada and the Ministère du Développement Durable de l'Environnement et des Parcs du Québec.

Distinction of environmental transport pathways using carbon-14 as a tracer in a wetland ecosystem (PO)

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A key aspect of decision-making for the purposes of environmental management is the necessity to trace contaminant emissions to source. Such information can provide critical insight into applying proactive measures or best practices to facility processes, activities, mitigation plans, or infrastructural design. To address this need, a field study was undertaken to characterize the spatial distribution of ^{14}C in *Sphagnum* moss as a tracer in a wetland ecosystem located down-gradient of a historical waste management area. In general, this study confirmed that the area of maximum ^{14}C concentration is highly localized in the wetland ecosystem, with relatively rapid declines in the mean and maximum specific activities, and the total areal coverage of the maximum ^{14}C contours for swamp surface vegetation, despite the long radiological half-life of ^{14}C. This suggests an overall decrease in ^{14}C influx into the swamp via groundwater and a net loss to the atmosphere over time. In addition, for the most part, moss sampling locations could be sub-divided into two groups based on changes in ^{14}C levels with distance from the site with the highest ^{14}C concentrations in the swamp. The first group represented locations that showed relatively higher ^{14}C concentrations and were adjacent to known areas receiving ^{14}C from groundwater inputs. The second group were more distant from source and likely received ^{14}C indirectly through atmospheric dispersion and deposition. Based on this assessment, it could be concluded that proximity to groundwater source, groundwater flow patterns, atmospheric transport, and habitat physical characteristics play important roles in the dynamics controlling ^{14}C distribution in wetland environments.

NATIONAL AGRI-ENVIRONMENTAL STANDARDS INITIATIVE

National Agri-Environmental Standards Initiative (NAESI): program overview (PL)

E. Roberts[1] and M. Bowerman[1]. [1]Environment Canada, Gatineau, QC

The common objective of agricultural sustainability is to maintain high agricultural productivity while protecting the environment and maintaining the quality of the land. To help guide the design of farm practices in achieving desired environmental outcomes, Environment Canada, in collaboration with Agriculture and Agri-Food Canada, over the past 4 years has developed a suite of national non-regulatory, science-based agri-environmental performance standards for certain variables in agricultural landscapes. National teams of scientists developed these standards in four themes: Air; Biodiversity; Pesticides; and Water. With the goal of defining healthy ecosystems on agricultural landscapes, our first task was to establish priorities for standard development. Of relevance to this conference, the Water Theme developed environmental benchmarks for nutrients, pathogens, sediments, instream flow needs, and water availability. Research was also carried out under the Pesticides Theme which focused on the top twenty in-use priority pesticides, as well as mixtures and risk-based performance standards. Approximately ninety performance standards specifying levels of environmental quality possible on agricultural landscapes with current technology (Achievable Performance Standards) as well as the desired levels of environmental quality (Ideal Performance Standards) in these altered landscapes were developed. Work on the NAESI standards is closely linked to other Canadian on-farm action programs, such as Watershed Evaluation of Beneficial Management Practices, and other performance measurement programs such as the National Agri-Environmental Health Analysis and Reporting Program. Thus, in addition to serving as scientific benchmarks to assess environmental quality, the standards will provide valuable information to direct future work on Beneficial Management Practices development or implementation.

Water quality of Canadian agricultural streams: defining nutrient concentrations to prevent eutrophication (PL)

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Inputs of nutrients (phosphorus, P, and nitrogen, N) to fresh waters can cause excessive aquatic plant growth, depletion of oxygen, and deleterious changes in abundance and diversity of aquatic invertebrates and fish. As part of a "National Agri-Environmental Standards Initiative" (NAESI), the Government of Canada committed to development of non-regulatory environmental performance standards that establish nutrient concentrations to protect ecological condition of agricultural streams. Analysis of data from long-term provincial monitoring programs and experimental studies in networks of 10-15 streams showed that agricultural land use increased nutrient concentrations in streams, resulting in increased sestonic and benthic algal abundance, loss of sensitive benthic macroinvertebrate taxa, and an increase in benthic diatom taxa indicative of eutrophication. Calculation of standards for total P or total N using three empirical methods followed by cross-calibration of these chemical standards with information on biological condition resulted in recommendations for total P and total N standards for agricultural streams in PEI, NB, southern QC, southern ON, south-western MB, and south-central BC. These recommended standards should result in good ecological condition with respect to benthic algal abundance, benthic diatom composition, and benthic macroinvertebrate composition. Research is continuing to determine whether these standards are protective of downstream receiving waters and to evaluate interactions of nutrients with other stressors (e.g., suspended sediments, pesticides), specifically their combined effects on aquatic food webs.

Effects of catchment disturbance on stream metabolism (PL)

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Metabolic activity of running waters is characterized by the ratio of primary productivity to community respiration (P/R) and indicates whether the energy supply of a particular stream is driven primarily through allochthonous or autochthonous inputs. Whereas undisturbed forested streams often derive energy through outside inputs, demonstrating higher rates of respiration than production, streams with disturbance to their riparian and catchment zones display increased rates of production over respiration. Our primary aim was to utilize detailed catchment and buffer zone disturbance features to further ascertain the effects of catchment disturbance on stream metabolism. Metabolism was measured using the *in situ* chamber method in five streams of varying levels of catchment disturbance (i.e. agricultural land use) in the spring, summer, and fall seasons in the Grand Falls region of New Brunswick. Other stream data, including riparian vegetation cover, turbidity, and temperature, were also examined to further understand variations in metabolism among the streams. Preliminary data analysis has revealed significant relationships between metabolism and buffer disturbance and temperature, and differences among seasons. Results of this research may help to clarify the impacts of land use disturbance on the energy supply and ecological condition of streams and further our understanding of stream ecosystem processes.

Flow characteristics of headwater streams across a gradient of agricultural land use and beneficial management practices (PL)

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Agricultural land use is a well documented source of stress to aquatic systems. Landscape clearing for agriculture has resulted in sediment and nutrient runoff to nearby stream catchment, and problems of soil loss from productive fields. In order to mitigate the effects of soil loss, beneficial management practices (BMPs) were developed to reduce runoff and nutrients loss to nearby watercourses. BMPs were found to be effective in the reduction of soil loss from fields, but little investigation has been conducted into the ameliorative effects of BMPs on stream condition. The purpose of this research is to investigate instream hydrograph patterns across a gradient of agricultural (%) and the implementation of BMPs. Headwater streams (catchment area: 1 to 4 km^2) were selected throughout the area, ranging in agricultural intensity. Hydrographs were created using daily discharge values over the ice-free season and imputed into the Indicators of Hydrologic Alteration program. Principal components analysis was conducted on the 32 IHA parameters to determine primary sources of variation in flow regimes. The component comprised of maximum flow events was found to account for the most variability (44 %) between stream land-use categories. By studying the hydrograph characteristics of streams across an agriculture and BMP gradient, we hope to see further advantages of implementing Beneficial Management Practices. We hope to investigate the merits of studying stream flow regime as an indicator of stream condition.

NAESI standards to prevent excessive sediment effects in Canadian streams (PL)

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Under the National Agri-Environmental Standards Initiative (NAESI), we were tasked with developing agri-environmental performance standards that will protect surface waters from excessive sedimentation. Sediment inputs derived from land-disturbing activities are a pervasive threat to stream ecosystems in North America and elsewhere. Of the many land-disturbing activities contributing to increased sedimentation to aquatic ecosystems, agriculture is the most common source in the US and Canada. We report on physical and biological standards that specify total suspended sediments, turbidity and deposited sediment thresholds predicted to be protective of desired ecosystem condition. This was accomplished by applying five approaches used for water quality standards development in Australia and New Zealand, Canada and the US to historical and contemporary regional data sets to compare their performance and to produce potential sediment standards. Across all regions of Canada, the abundance of pollution-tolerant taxa increased as habitat was degraded by sediments. This caused a significant reduction in several metrics of ecological quality, including measures of benthic macroinvertebrate diversity (total invertebrate and EPT richness) and EPT relative abundance. Future methods development for setting standards in agricultural watersheds should continue along several investigative paths, including the use of biological endpoints to establish standards. In addition, development efforts should attempt to link catchment topography, land use, and hydrological response to in-channel sediment transport, deposition, and biological condition.

A methodology for the estimation of achievable performance standards for nutrients and sediments in streams draining agricultural watersheds (PL)

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For the National Agri-Environmental Standards Initiative (NAESI), a non-point source water quality model (Soil and Water Assessment Tool – SWAT) was used to develop achievable performance standards (APS) for nutrients and suspended sediments (TSS) in streams of two agricultural watersheds, the Black Brook watershed (BBW), New Brunswick, and the Raisin River watershed (RRW), Ontario. For the BBW, simulations of BMP implementation (including diversion terraces, crop rotation, tillage practices, and fertilizer application rates) forecast reductions in average concentrations of TSS by 92 %. The provisional average daily APS value for BBW was 56.7 mg·L^{-1} for TSS. For nutrients, provisional APS for average daily concentrations of nitrate-N and soluble P were 2.13 and 0.010 mg·L^{-1}, respectively. Compared to current conditions, the impacts of these BMPs on water quality are greater for sediments and soluble P than nitrate-N. For the RRW, simulations of "ideal" and realistic biodiversity conservation scenarios (including changes to extent of forest and wetland coverage, and buffer zone widths) forecast that TSS would be reduced by 12.8 % and 2.6 %, respectively, in comparison with current conditions, and that APS levels of 1.78 $mg \cdot L^{-1}$ for TN and 0.031 $mg \cdot L^{-1}$ for TP could be achieved. APS for both watersheds are likely to change as additional BMPs are incorporated into the modeling framework (i.e., grassed waterways and riparian management for BBW, and tile drainage management and restriction of livestock access for RRW). While this modeling approach enables determination of APS for watersheds that are specific and context dependent, meaningful scenarios and forecasts are predicated on the availability of robust input data, water quality data, and estimates of BMP efficacy.

National water quality surveillance study for waterborne pathogens in vicinity of experimental agricultural watersheds (PL)

T. Edge[1], R. Kent[2] and R. J. Phillips[2]. [1]Environment Canada, Burlington, ON [2]Environment Canada, Gatineau, QC

The National Agri-Environmental Standards Initiative (NAESI) began in 2004 to develop, field-test and recommend science-based non-regulatory environmental performance standards for Canadian agriculture management practices. NAESI standards themes include biodiversity, pesticides, and air and water quality. Under water quality, standards are currently being developed for nutrients, sediments, instream flow needs, and waterborne pathogens. At the outset, an analysis was undertaken of current indicators used in determining occurrence, quantity, source, and relative risks of waterborne pathogens to human and non-human receptors. With that information in hand, the national team then embarked on Canada's first national waterborne pathogen surveillance program, focussing on detecting and quantifying a broad suite of relevant agriculturally-derived protozoan and bacterial pathogens along with various water quality indicators--microbial, chemical, and physical. In order to account for waterborne pathogens from non-agricultural sources like wildlife, a maximum natural background level of pathogen diversity was determined from NAESI reference sites in the test watersheds. These reference sites were located to best reflect a zone of non- or minimal-agricultural influence. Since a close association was found between increasing annual mean values of pathogen diversity and *E. coli* concentration at agricultural sites, a NAESI pathogen standard was derived in terms of the *E. coli* concentration corresponding to the maximum natural background pathogen diversity expected at agricultural sites. Several Canadian experimental agricultural watersheds posing high pathogen risk to water quality and water uses were selected as research study areas. These watersheds have areas of Intensive Livestock Operations (ILO's); specifically, intensive dairy, beef, fowl, and pork. Surveillance data from 2005-06 and 2006-07 suggest some challenges in expressing pathogen potential based on existing standard indicators such as *E.Coli*. For, as ILO and pathogen sources vary, so too do predictive indicators or combinations thereof. Final recommendations of relevant standards (indicator suites) will require further testing and application as national water quality performance benchmarks in agricultural waters. This platform session will outline some of the surveillance results as well as some of the key factors in the development and testing of promising indicators for agricultural waterborne pathogens.

Overview of NAESI Pesticide Theme (PL)

P. B. Jiapizian[1] and M. J. Demers[1]. [1]Environment Canada, Gatineau, QC

Pesticides are widely used in agriculture to control pests and therefore decrease damage to crops and maximize yields. Pesticides may, however, create risk to the environment and human health. The goal of the Pesticide Theme, within NAESI, was to produce a suite of pesticide standards that will serve as benchmarks of environmental quality in agricultural settings. These standards may inform and guide future efforts in the reduction of environmental risks posed by the use of pesticides in agriculture. Over the last four years, the Pesticide Theme has produced approximately 60 standards in the various subject areas:

- Ideal Performance Standards (IPS) (acute and chronic) have been calculated for 20 pesticides in water and one pesticide in sediment;
- Achievable Performance Standards (APS) have been calculated for six test watersheds;
- A risk-based approach was used to develop protection limits for mammals, birds, pollinators and invertebrates against acute pesticide exposures and to protect birds against chronic pesticide exposures;
- An approach for the determination of a Commodity-Based IPS (CB-IPS) was developed and tested in potato producing regions of Canada;
- An approach for the determination of a mixtures IPS was developed and tested in Prairie wetland ecosystems; and
- A meteorological spray advisory was developed to guide pesticide application.

This presentation will provide a brief review of the standards and the methods which were used to derive the standards under the Pesticide Theme of the NAESI program.

TAKE A LEAP OR BUILD A BETTER BRIDGE: CORROBORATING, EXTRAPOLATING AND PREDICTING ADVERSE EFFECTS BETWEEN THE LABORATORY AND FIELD

Ecology comes first in ecotoxicology (PL)

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Ecotoxicology is, by definition, not simply environmental toxicology. It incorporates adequate ecological knowledge to test appropriate taxa using appropriate endpoints and exposures: to protect ecosystem services, in particular essential populations and communities, from toxic exposures; and, to this end, to extrapolate from a few to many species and allow appropriate extrapolation from the laboratory to nature. Testing can range from populations and communities to individual organisms, to organs, to cells, to enzymes, so long as ecological relevance is established, ideally *a priori*. Further, testing needs to be designed and interpreted considering toxicity-modifying factors (not only physical and chemical but also biological [e.g., behavior]) and the possibility not only of direct, but also of sometimes much more significant indirect effects (to competitors, predators, and prey – e.g., trophic cascades). Vulnerability to toxic chemicals can change relative to global environmental changes, nutritional status, pathogens, and other stressors. Toxicity testing using laboratory surrogates is not necessarily ecotoxicology unless those surrogates have been adequately compared to critical ecological processes and not found wanting. The ecologically most appropriate test organisms (including most vulnerable life stages and populations), endpoints, and exposure routes may not always be intuitively obvious or easy to test, but they will provide the most relevant and useful information for protecting ecosystem services.

Co-ordination of integration: how a multi-stakeholder watershed monitoring program has built bridges across the Thompson River (PL)

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The Thompson River has been described as the most studied river in North America. This river has important fisheries resources, in particular steelhead and salmon. In the early 1970s several problems occurred within a short period of time: excessive algal growth, discolouration of the water, foaming, fish tainting, and decreased diversity of the benthic invertebrate community. There were concerns that the fisheries resources of the Thompson River would be severely compromised. As a result, research was conducted on the river throughout the 1970's and 1980's to determine the cause of these problems and to reduce the risk of these conditions recurring. Although these studies have been completed, monitoring continued on the river by several government agencies and industrial and municipal dischargers. Each monitoring program had its own goals and objectives and was not integrated with the other programs, resulting in duplication and incompatible data. In recognition of the importance of the Thompson River and the limited resources available, the stakeholders determined a need for the integration of the separate programs into a single, coherent monitoring program. The stakeholders included government agencies (federal, provincial, and local), First Nations, dischargers and their consultants, residents, and interest groups. The integrated monitoring program was initiated in 2004 and will continue to 2011. This presentation will summarise the challenges which were faced in initiating this co-ordinated approach and how integration has worked to build bridges with the stakeholders along the Thompson River. A summary of the first three years' worth of data will also be presented.

Fish lifecycle exposures: Bridges to effects in wild fish (PL)

J. Parrott[1], *G. Tetreault*[1] *and M. McMaster*[1]. [1]*Environment Canada, Burlington, ON*

Predicting and assessing effects in wild fish from standard laboratory tests is difficult. Standard short fish exposure bioassays developed in the 1970s and 1980s work well for assessing acute toxicity and immediate effects of compounds and effluents. In Canada, fortunately, many of the effluents we regulate are no longer acutely toxic. Some compounds, effluents and discharges, however, do have long-term impacts on fish health. To assess the effects of long term exposures, we have used the fathead minnow lifecycle bioassay for several pure compounds and mixed effluents. Lifecycle tests encompass all "critical windows" of exposure: egg, larvae, developing and maturing juvenile, reproduction of adult fish, and survival of the F1 generation. Fish lifecycle exposures to the synthetic estrogen used in birth control pills, ethinyl estradiol (EE2), show thresholds of effect (on F1 egg fertilization success) in the 1 $ng \cdot L^{-1}$ range. This lab-derived cutoff mirrors responses to EE2 in wild minnows. Lab lifecycle exposures to municipal wastewater effluents (MWWE) show changes in secondary sex characteristics and decreased breeding, despite increased growth. Wild fish exposed to MWWE show similar effects on sex characteristics. Effects of pulp mill effluents on fish growth and reproduction in lifecycle studies also mirror effects seen in some wild fish. The lifecycle tests have the disadvantages of being expensive and lengthy. However, for mimicking effects of real environmental exposures and for use in risk assessments, these tests provide valuable data that are difficult or impossible to obtain using shorter lab fish exposures.

Investigations into the potential impacts of oil refinery effluent on fish, conducted in the field and laboratory (PL)

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Investigations into the potential toxicity of oil refinery effluent on adult fish were initiated in 2003 at the largest oil refinery in Eastern Canada (throughput of 300 000 barrels·day^{-1}). The refinery discharges its effluent at a rate of 24,274 m^3·d^{-1} into Little River, an estuarine stream that opens into the harbour in Saint John, New Brunswick. Initial studies found a decrease in fish abundance and species richness in the downstream environment that was attributed to intermittent depressions in dissolved oxygen associated with periodic ballast water discharge. The most recent study was initiated to evaluate potential recovery of fish populations following reductions in ballast water discharge. Field surveys (2005-2007) have indicated no recovery in the fish community and a slight improvement in dissolved oxygen. An increase in nutrient levels was also noted. Caged bioassays with mummichog (*Fundulus heteroclitus*, a brackish water fish) and northern redbelly dace (*Phoxinus eos*, a freshwater minnow) displayed increased liver size and liver detoxification enzyme activity (ethoxyresorufin-*o*-deethylase) in mummichog; dace did not survive in adequate numbers in the caged bioassays for statistical analyses, and effluent toxicity was indicated. Laboratory fish bioassays with effluent and sediment show increased liver detoxification enzyme activity in treatments with sediment from Little River below the effluent outfall. These results indicate that historic sediments in Little River play a contributing role, amongst other factors, towards the decrease in fish abundance and species richness.

Comparative analysis of response patterns between laboratory and field mesocosm studies (PL)

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Artificial stream mesocosm bioassays using various technologies have been conducted across Canada in both laboratory and field settings. Research has been conducted largely in freshwater habitats, exposing fish and benthic invertebrates to a variety of stressors including sewage, mining (base metal and uranium), and pulp and paper effluents in isolation and in combination. The intention of these studies has been to use experimental manipulations to define biological response patterns to complex mixtures as well as to isolate source (different process streams, causative metals) of effects, to prioritize contribution of different sources in multiple stressor environments, and to examine different exposure pathways (water, diet, sediment). Studies have been conducted in both field and lab environments, often using the same stressor to allow for comparative analysis of results. A review of these studies will be presented.

The improvement of Environment Canada's echinoid fertilization assay for porewater testing (PL)

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Since its publication in 1992 and formal amendment in 1997, Environment Canada's (EC's) "Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars), EPS 1/RM/27" has been used on a regular basis in the Disposal at Sea Program, which falls under the authority of the Canadian Environmental Protection Act. Under this program, this sediment porewater method is one of a battery of tests that can be used to help evaluate the suitability of dredged material for open water ocean disposal. After 15 years of application, however, it was recognized that specific aspects of the test method needed to be re-evaluated. In particular, the echinoid fertilization test gave results inconsistent with a known sediment contaminant gradient in Sydney Harbour, NS, and inconsistent with the results produced using other sediment toxicity tests. False positives with "clean" reference sediments were also reported. EC decided to investigate the improvement of the porewater-testing component of the echinoid fertilization assay through an inter-laboratory study comparing results obtained using their own method and those resulting from an echinoid fertilization assay developed at the USGS Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas. The objective of the study was to determine whether the echinoderm method from MERS would yield more relevant results with sediment porewaters that have been shown to be problematic using EC's echinoid fertilization assay. The results of the inter-laboratory study and the scope of changes to EPS 1/RM/27 will be discussed.

Digging down to the core of boreal forest soil tests (PL)

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Development of standardized eco-toxicological methods for testing of contaminated soils has advanced significantly in Canada in the last 10 years. At least 15 species are offered as test organisms, including invertebrates and plants. At present, the soil collected for testing is typically processed by digging, drying, sieving and re-wetting before test organisms are added to it. This presentation will describe use of intact soil cores in comparison with this traditional method of soil handling and preparation. Six species of boreal forest plants were grown in parallel tests at two sites in Alberta, one contaminated with weathered crude oil and one with salt. Severe toxic effects at the salt-contaminated site observed in soil cores tests were confirmed in definitive dilution tests, as were the less severe effects seen at the hydrocarbon-contaminated site. Soil cores at two reference sites were also tested with boreal forest plants. Although plants did not grow in cores to the same size as in the processed soil, these tests have potential use in locating 'hot spots' of contamination without disturbance of soil horizons and indigenous organisms. They also have the advantage of speed, as tests can be initiated immediately. Standardization of the boreal forest plant toxicity test is proceeding. A proposed method for testing of contaminated soil using boreal forest plants grown in intact soil cores is presented.

Weighing out ecosystem stress: application of stable isotopes as a biomarker for starvation stress in natural ecosystems--a molecular to ecosystem approach (PL)

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Natural fluctuations in aquatic ecosystems, such as those associated with species introductions, can serve as an environmental 'baseline' against which to compare the potential impacts of anthropogenic activities relative to those inherent to the ecosystem under consideration. Through such considerations, environmental assessors are able to evaluate the potential magnitude of stresses that can be introduced into ecosystems through anthropogenic activities (such as exposure to contaminants) relative to unrelated system processes (e.g., natural population dynamics). For example, the introduction of an efficient predator into a small aquatic system can lead to fish dietary shifts due to prey depletion, and in extreme cases, declines in fish condition to the point of starvation, reductions in biodiversity, and corresponding changes in ecosystem structure and function. Resultant changes in prey availability can subsequently influence the transfer of contaminants and the corresponding exposures received through dietary pathways. Changes in the foodweb structure of Perch Lake, a small lake receiving radionuclide inputs from upstream sources over a 50-year period, were studied following the introduction of a keystone predator (northern pike) using stable isotopes as tracers. Shortening of the Perch Lake food chain occurred in the post-pike fish community, with significant declines in δ^{15}N signatures of the top predator over time, due to depletion of the prey base. Based on an evaluation of stable isotopes, radionuclide exposure and effects data, reductions in the health of the Perch Lake ecosystem could be not linked to radiological exposure. Instead, these changes were related to the introduction of northern pike and the subsequent trophic cascade in the Perch Lake fish community.

Using the rainbow trout (*Oncorhynchus mykiss*) RTL-W1 liver cell line as a model for *in vitro* ecotoxicogenomic analysis of the effects of pulp and paper mill effluent exposure in fish (PO)

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Canadian pulp and paper mills are required to regularly test their effluents for potential toxicity to aquatic wildlife. While fish are ecologically and biochemically ideal organisms for this routine monitoring, their use is costly and increasingly impractical. The use of cultured cells likely represents a good alternative to animals for this work. Compared to fish, a fish liver cell line is easier to maintain, is less costly, requires shorter exposure periods, allows more replicates and thus increased numbers of biological samples per experiment, and permits toxicant exposure biomarkers to be assayed in both the cells and the culture media. Due to its single tissue type, a liver cell line also facilitates understanding of the pathways involved in molecular responses to toxicant exposure. On the other hand, cultured cells lack the interactions present between different tissues and complicate obtaining physiologically and ecologically relevant information. To gauge whether RTL-W1 rainbow trout liver cells could serve in this capacity, we are using real-time PCR to characterize the expression of several genes responsive to endocrine disrupting compounds and oxidative stress in RTL-W1 cells exposed to pulp and paper mill effluents. Our goal is to compare these results to those from livers from whole fish, and use this information to better understand how pulp and paper effluents affect wildlife at the cellular level.

The problem with *Lemna* is minor: an examination of duckweed's ability to predict effects in field tests (PO)

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The floating macrophyte *Lemna* spp. (duckweed, typically *L. minor*) is the only currently required aquatic plant test for the registration of plant protection products in Europe and North America. For almost the past decade there has been a push to expand the number of species included in the risk assessment process beyond just *Lemna* spp. The reasons given range from: 1) *Lemna* being a monocot and therefore not protective of dicots to certain chemicals, 2) it does not interface with the sediment and have an extensive root system, meaning this route of exposure is unaccounted for in current lab testing, to 3) duckweed being a plant that thrives in eutrophic conditions, and would not be representative of plants in meso- or oligotrophic conditions. These can be summed up as 'lab results do not predict field observations', but is this really the case? This review examined this contention by comparing *Lemna* data from laboratory bioassays to data from those studies conducted in the field, usually micro- or mesocosm, for the same compound. Other macrophytes within the field test systems were compared to Lemna spp. responses where possible to get an understanding of its relative sensitivity. The general observations were that laboratory data were within an order of magnitude of field data, and in many cases, actually more sensitive. It would appear that *Lemna* does a reasonable job of protecting aquatic plants under field conditions in the current framework, and at this point, there is no strong need to widely expand the array of aquatic macrophytes tested at lower tiers of the risk assessment.

CUMULATIVE EFFECTS ASSESSMENT

Perspectives on cumulative effects assessment: toward a watershed-based approach (PL)

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The need to better manage the cumulative environmental effects of human activities on Canada's watersheds is well argued, but the practice of watershed-based cumulative effects assessment (CEA) has been slow to evolve. In those cases where CEA has been done, it has been informed largely by project-driven impact assessment thinking--focused on describing the current state of the watershed or modeling system response to human-induced stressors, rather than also on projecting trends, scenario building, and discerning desirable futures. The result is a type of CEA that is narrow, reactive, divorced from the broader watershed-planning context and, overall, ill-equipped to deal with cumulative environmental change. In this presentation we step back from discussions of the science of CEA and examine more closely the underlying nature of CEA from two broad perspectives: environmental impact assessment-driven or stressor-based CEA, and regional environmental assessment or effects-based CEA. In doing so, we identify several constraints to current CEA and suggest a number of requisites to moving forward. We argue that 'good' watershed CEA requires more than simply expanding traditional project-based impact assessment thinking and practice to a broader spatial scale; it represents a different way of approaching the interrelationships between human-induced stressors and cumulative environmental change.

Examining cumulative effects in watersheds dominated by intensive urban and agricultural activities in B.C. (PL)

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The relationship between percent imperviousness and macro-invertebrate diversity has often been used as a good indicator of cumulative effects of pollution in urban watersheds. Our recent research showed that traffic density, which is one of the major sources of pollution, needs to be included in such assessments. In agricultural watersheds, the calculation of annual nutrient balances, the determination of livestock density, and the chemical analysis of sediments are also proving to be good indicators of cumulative effects. Since B.C. established an agricultural land reserve (ALR), urban expansion is occurring primarily in the upland portion of the coastal watersheds. This means that the combined impact of urbanization and intensive agriculture is emerging as a major issue in many watersheds in the Lower Fraser Valley in B.C. To address the cumulative effect of these combined activities in the same watershed requires the use of a GIS-based land-use analysis in combination with surveys on traffic intensity, livestock numbers, and use of agrochemicals. Using exchangeable resins in the form of Diffuse Gradient Thin Film (DGT's) to determine bioavailability of metals and other pollutants offers the possibility to determine relationships between land-use indicators and their effect on the aquatic biota without having to resort to labor-intensive analysis of the aquatic biota. Given the complexity of cumulative effects, a combination of indicators is likely most effective and should include quantitative data on land-use changes and annual loading estimates of sediments and pollutants.

Identification of water quantity and quality trends contributing to cumulative effects in the Athabasca River Basin (PL)

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Novel approaches addressing aquatic cumulative effects over broad temporal and spatial scales are required to track changes and assist with sustainable watershed management. Cumulative effects assessment (CEA) requires the assessment of changes due to multiple stressors both spatially and temporally. The province of Alberta, Canada, is currently experiencing significant economic growth as well as increasing awareness of water dependencies. There has been an increasing level of industrial, urban, and other land-use related development (pulp and paper mills, oil sands developments, agriculture, and urban development) within the Athabasca River basin. Much of the historical water quantity and quality data for this basin have not been integrated or analyzed from headwaters to mouth, which affects development of a holistic, watershed-scale CEA. The main objectives of this work were to 1) quantify spatial and temporal changes in water quantity and quality over the entire Athabasca River mainstream across pre-development (1966-1976) and current day (1996-2006) time periods, and 2) to evaluate the significance of any changes relative to existing benchmarks (e.g. water quality guidelines). Data were collected from several federal, provincial and non-government sources. A 14-30 % decrease in discharge was observed during the low-flow period in the second time period in the lower three river reaches, with greatest decrease occurring at the mouth of the river. Dissolved sodium, sulphate, chloride and total phosphorous concentrations in the second time period were greater than, and in some cases double, the 90th percentiles calculated from the first time period in the lower part of the river. Our results show that significant changes have occurred in both water quantity and quality between the pre-development and current-day Athabasca River basin. It is known that in addition to climatic changes, rivers which undergo increased agricultural, urban, and industrial development can experience significant changes in water quantity and quality due to increased water use, discharge of effluents, and surface run-off. Using the results from this study, we can begin to quantify dominant natural and man-made stressors affecting the Athabasca River basin as well as place the magnitude of any local changes into an appropriate context relative to trends in temporal and spatial variability.

Cumulative effects assessment of South Saskatchewan River basin headwater rivers (PL)

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The development of Alberta's 'Water for Life' water management strategy and closure of the Oldman and Bow basins to new water licenses underscores that pressure on water resources in Southern Alberta is high. Cumulative Effects Assessment is a potential mechanism by which the impact of land use and cover, plus point sources, might be identified throughout Southern Alberta and used for future water management. We have created a GIS to spatially organize land cover and use, and are coupling this to measured instream metrics of water quantity and quality. We have found that major urban centers have footprints that extend 100-150 km downstream of wastewater inputs, and that different land use in the three major sub-basins leads to different stresses as identified by effects assessments. Highlights of our findings will be presented, as well as current limitations to completing a comprehensive cumulative effects assessment.

A holistic approach to the integrated assessment of the health of river basins: a framework for the St. John River, New Brunswick, Canada (PL)

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Integrated watershed management is challenged by fractured political responsibility and hindered by the absence of a comprehensive ecosystem-based framework for evaluating sustainability. Ecological integrity and sustainability are inherently valuable, but existing watershed efforts usually focus primarily on protecting human health and the human use of ecological resources. Integrating ecological services into watershed management requires an understanding of the accumulated environmental state, the assimilative capacity of the receiving systems, and the potential consequences of future developmental scenarios and climate changes. In 2001, The Canadian Rivers Institute (CRI) was formed as an open collaborative network aimed at understanding the ecology of river systems. The CRI focuses many of its collaborations on the St. John River, and a major initiative is the development of a framework for understanding the assimilative capacity of the St. John River system. The evaluation framework represents an effects-based approach to monitoring, assessment, and priority-setting that is aimed at understanding the factors limiting ecosystem services. The St. John is a 700-km long 7th-order river system that crosses the international border between the US and Canada in the upper third of the basin and forms the border between Canada and the US for >50 km. The research effort on this watershed has grown into a multi-institutional partnership including federal and provincial government agencies, private industry, and NGOs in a non-competitive, inclusive model that operates without core funding. Community stakeholders play an increasingly important role in the process, and the studies are attempting to include socioeconomic assessment. This presentation will present the key assumptions, data needs, and friction points for site-specific adaptation of the framework to a watershed.

Contrasting responses of blacknose dace (*Rhinichthys atratus*) and slimy sculpin (*Cottus cognatus*) exposed to municipal and industrial effluents in the Upper St. John River (PL)

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Three basic characteristics are required of any species used in an environmental effects monitoring program: abundance, a favourable distribution, and site fidelity. The characteristics of abundance and distribution are easy to determine, but site fidelity is more difficult. Methods, including PIT tagging, have been used in the past to identify site fidelity, but they can be limited by the size of fish that can be tagged. In Edmundston, NB, several sewage and pulp and paper mill outfalls enter the St. John River in close proximity to each other, increasing the need for confident estimates of site fidelity of sentinel species. In our study, we examined stable isotopes signatures in blacknose dace, slimy sculpin, and their invertebrate prey for their response to the effluents and their site fidelity (with stable isotopes) and feeding relationships. The slimy sculpin collected downstream of sewage inputs had increased condition and different carbon and nitrogen isotopes when compared to those collected upstream, while dace showed no change in condition and an overlap in their isotopic ratios versus those caught at the reference site. The food web data showed isotopic shifts in some invertebrates, but not in others collected downstream of the sewage inputs. Our data show that nutrient enrichment responses are asymmetric across fish species and that this can be related to impacts on the fish's food base and changes in food preference.

Teasing apart the cumulative effects of multiple stressors (PL)

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Cumulative effects assessment has a long but imperfect history in environmental impact assessment. Why imperfect? Most cumulative effects assessments are descriptive rather than quantitative. Despite thousands of studies, statistical tools to tease apart cumulative effects are generally lacking. Recent interest in multiple stressors has underscored the need to quantitatively evaluate the cumulative effects of two-or-more stressors. We propose that appropriate statistical tools exist and are widely used in aquatic community ecology. With data collected in a collaborative study of mines and reference sites in northern Ontario, we use multivariate multiple regression, or redundancy analysis, to partition variation from three distinct sources. That is, variation in four benthic community metrics is partitioned among natural reference-site habitat features, mining effluent and tailings impacts, and uncontrolled urban land-use effects. This approach quantifies the individual and combined effects of different factors that potentially affect the benthic community, providing a significant quantitative tool for cumulative effects assessment and permitting an objective evaluation of the separate impacts of multiple stressors.

ADVANCES IN ENVIRONMENTAL CHEMISTRY

Total phosphorus analytical methods for measurement of low and trace concentrations in oligotrophic and mesotrophic lakes (PO)

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The study and prevention of eutrophication of oligotrophic and mesotrophic lakes of the province of Quebec requires knowledge of the actual phosphorus concentration in those lakes in order to arrive at precise and valid eutrophication evolution indicators. The total phosphorus measurement precision of concentrations less than 10 $\mu g \cdot L^{-1}$ becomes a major factor. The CEAEQ has developed two automated methods by persulfate extraction and colorimetric measurement with method detection limits (MDL) of 2 $\mu g \cdot L^{-1}$ and 0.6 $\mu g \cdot L^{-1}$. The first method, called *trace phosphorus*, uses decontaminated bottles and materials. The precautions taken during sampling and samples treatment in order to avoid contamination resulted in obtaining a MDL as low as 0.6 $\mu g \cdot L^{-1}$. The second method, with a MDL of 2 $\mu g \cdot L^{-1}$, doesn't use decontaminated materials and is used for low and medium phosphorus concentrations. The *Direction du suivi de l'état de l'environnement*, from the *Ministère du développement durable de l'Environnement et des Parcs*, has been studying phosphorus concentrations in the province of Quebec's lakes for many years. In summer 2008, those two methods were used to analyse phosphorus in more than 300 lakes throughout Quebec. The low MDLs are useful to study with precision phosphorus in oligotrophic and mesotrophic lakes.

Using tracer elements to estimate sediment content in prey species collected in the St. Lawrence Estuary beluga whale habitat (PO)

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Ingestion of contaminated sediments associated with benthic prey species could be a significant source of exposure of beluga whales or other benthic predators to toxic compounds. The objective of this study was to identify tracer elements that could be used to estimate quantitatively the sediment content in various prey items or to evaluate sediment ingestion in benthic predators. A variety of pelagic and benthic prey species (9 species of fish and invertebrates) and sediments (from three coastal sites) were collected in the St. Lawrence beluga whale's habitat in the Saguenay Fjord and in the St. Lawrence Estuary. Concentrations of more than 30 elements were determined by ICP-MS. A number of these elements, most particularly lanthanides (rare earth elements) were identified as promising sediment tracers. These elements were barely detected in pelagic organisms but were detected in whole non-depurated benthic organisms and in sediments. Shrimp, plaice and tomcod appear to have the highest sediment content among the different prey tested. These tracers will be used to better characterize dietary exposure of benthic predators to sediment contaminants and to investigate adverse health effects associated with such exposures.

NANOTOXICOLOGY

Eco-toxicogenomics of CdTe quantum dots in rainbow trout (PO)

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The purpose of this study was to examine the toxic effects of cadmium-telluride (CdTe) quantum dots on gene expression in rainbow trout (*Oncorhynchus mykiss*). Rainbow trout (4-6 g) were exposed to increasing concentrations of CdTe (0, 1.0, 1.8, 5.6 $\mu g \cdot L^{-1}$ Cd) and cadmium sulphate ($CdSO_4$, 0, 0.56, 1.0, 1.8 $\mu g \cdot L^{-1}$ Cd) for 96 h at 15°C. Total RNA was extracted from liver tissue and reverse transcribed to cDNA. A two-channel reference design was used, in which Cy5-labelled cDNA and a Cy3-labelled universal DNA reference (UDR) were hybridized to 207-gene rainbow trout cDNA microarrays. Characterization of CdTe (proportion of particulate versus molecular form of Cd) was also conducted. Analysis of the microarray data showed effects of CdTe and $CdSO_4$ on the expression of several classes of genes, including binding/transport, endocrine, immune, metabolism, oncogene, proteolysis, signal transduction, structural and transcription. A total of 43 genes responded to only CdTe, 13 to only $CdSO_4$, and 8 to both CdTe and $CdSO_4$. Biomarkers of exposure to estrogenic compounds such as vitellogenin and vitelline envelope proteins were upregulated in rainbow trout exposed to CdTe but not in those exposed to $CdSO_4$, indicating metallo-estrogenic effects of CdTe. Validation of the microarray data by reverse transcription-quantitative PCR (RT-qPCR) is in progress. The metallo-estrogenic effects of CdTe in particular are being examined. In conclusion, the gene expression profile of rainbow trout exposed to CdTe differs from those exposed to $CdSO_4$, allowing the identification of biomarkers and potential mechanisms of toxicity of the particulate versus molecular form of cadmium.

Assessing the potential immunotoxicity of nanoparticles in channel catfish using high-throughput techniques (PO)

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The potential toxicity of manufactured nanoparticles (NP) is of growing concern since they will undoubtedly find their way into the environment. We examined the effects of water-soluble NP exposure on immune function in channel catfish (*Ictalurus punctatus*) cell lines using high-throughput microplate and flow cytometric based assays. The use of cell lines allows for the examination of interactions between specific immune cell types, including B and T cells, natural killer cells, and monocyte/macrophages. We assessed the effects of silica quantum dots and helical rosette nanotubes on cellular viability, proliferation, antioxidant pathways, and other immune cell functions. These studies are designed to determine the relative applicability of using immune cells as high-throughput indicators of the general and specific mechanisms of NP toxicity. The goal of the study is to provide feedback to NP engineers to facilitate the development of safer NPs while preserving the unique and beneficial properties of these materials.

SENSORY SYSTEMS: BRIDGING THE GAP BETWEEN BEHAVIOR AND ECOTOXICOLOGY

Habitat preferences of mud snails, *Ilyanassa obsoleta*, in response to stress (PO)

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The mud snails (*Ilyanassa obsolete*) inhabit the mud flats and salt marshes of the Minas Basin, Bay of Fundy, Nova Scotia, Canada. These gastropods are primarily deposit feeders and can be easily seen at low tide in varying density depending on the time of year and location of the beach. These snails and other related species are often present near small streams potentially receiving some agricultural runoff from fields treated with biocides. They are also abundant near urbanised locations, where they are found on the surface of rocks and pilings of dockyards. Our interest focused on examining the habitat requirements of the animals, as much in terms of maintenance in seawater as in sediments and on the role played by the presence of food relative to that of freshwater, pesticides or harbour derived contaminants. The survival, horizontal and vertical movement of the marine snails described as an avoidance/preference response relative to spiked/reference sediments or seawater was investigated. Examples of the results will highlight the knowledge gained about the stress response or tolerance of this invertebrate relative to exposure conditions.

Real-time effluent biomonitoring: the toxicity early warning system (TEWS) (PO)

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Like the canary in the coal mine, Lakehead University has developed a system that utilizes rainbow trout *(Oncorhynchus mykiss)* to detect acute levels of toxicity in various industrial effluents: The Toxicity Early Warning System (TEWS). With the co-operation of the Pulp and Paper Industry in Northwestern Ontario the TEWS is becoming a crucial component in the monitoring of effluent toxicity prior to its release into the environment. Employing fish sensors, the TEWS detects the presence of a broad range of toxic substances. Changes in fish ventilatory behaviour and certain locomotion activities are detected instantly through noninvasive electrode sensors in each tank. Types of responses measured include: changes in whole body movement, breath depth, opercular rate and gill purging, which are all stress indicators. Fish signals are amplified, filtered and stored digitally on a computer for statistical analysis. Present TEWS results indicate that changes in ventilatory behaviour has wide potential application as a biomonitoring tool for industry because (a) a wide array of chemical substances generate behavioral responses, (b) the behavioral responses are rapid and sensitive, and (c) the level of behavioral response occurs in proportion to the toxicant concentration. In combination with effluent quality standards for chronic exposure, the TEWS allows for healthy fish populations to potentially coexist with the byproducts of industrial activities.

Movement patterns of northern pike (*Esox lucius*) within gradients of uranium mining effluent discharges (PO)

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Anthropogenic activities such as metal mining can alter watershed hydrology and fish habitat of downstream areas. Understanding how variation in habitat and water characteristics influences freshwater fish movement, distribution, and home range is crucial to ensure the sustainability of fish populations. The objective of the present study was to determine the movement rate, location fidelity and home range of adult northern pike (*Esox lucius*) within gradients of uranium mining effluent discharges. Fish were collected from a reference site and an exposure site located downstream of effluent discharge. Digitally coded radio-tags were surgically implanted into the fish body cavity following sex, weight and length evaluations. Fish locations were seasonally recorded between September 2004 and May 2006 using a Lotek SRX_400 receiver with an attached Yagi antenna. Preliminary results suggest that tagged pike did not migrate out of the study area during the tracking period, with the mean distance traveled ranging from 400 to 600 m. Data analysis comparing the distances traveled and home range between reference and exposure site fish as well as sex differences in habitat use is in progress.

ERRATUM

The following paper was inadvertently omitted from the Proceedings of the 34th Annual Aquatic Toxicity Workshop , Halifax, NS

A Critical Review of the Environment Canada *Lemna minor* Biological Test Method EPS 1/RM/37 – March 1999

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Aquatic macrophytes are important species in aquatic environments because they influence the structure and function of aquatic ecosystems. They can define predator-prey relationships, are important in nutrient cycling, alter the physical environment, and can determine the level of phytoplankton biomass (Scheffer and Jeppesen 1998). As such, an understanding of their susceptibility to environmental contaminants should be an essential component of toxicity assessment. This has been recognized by the Canadian Council of Ministers for the Environment (CCME) through their inclusion of macrophytes as a key component in the establishment of Environmental Quality Guidelines (CCME 1999). To standardize the use of macrophytes in toxicity assessment, Environment Canada developed the standard bioassay method EPS/1/RM37 (the Test Protocol) for the determination of toxicity using the aquatic 'duckweed' *Lemna minor*. The purpose of this study was to critically evaluate several components of the Test Protocol standard method against fundamental concepts and understandings of toxicity assessment and duckweed biology, with particular reference to the effect of methodology on the assessment of metal toxicity. Components of the Test Protocol that were examined include light requirement, response variables, nutrient media, and choice of species. In addition, the Environment Canada method was compared with the recently released method developed by the Organization for Economic Cooperation and Development (OECD 2006).

The Environment Canada Test Method specifies maintenance of a test-condition light level of between 63 to 72 $\mu mol \cdot m^{-2} sec^{-1}$. This is slightly lower than the OECD requirement of 85 to 135 $\mu mol \cdot m^{-2} sec^{-1}$. However, both of these light levels approach the reported light compensation point for duckweeds, of approximately 40 to 60 $\mu mol \cdot m^{-2} sec^{-1}$ (Landolt and Kandeler 1987). The result is that duckweeds grown according to the Test Protocol will tend to exhibit slow and inconsistent growth. The slow rate of growth under the specified Test Protocol light intensity is apparent given that the acceptable threshold level for growth rate within the Test Protocol is a doubling time of only 2.33 days (56 hours). By contrast, published growth rates for floating duckweed species suggest that maximal growth rates produce doubling times that are in the range of 20 to 24 hours (Landolt and Kandeler 1987). A seven-day test with a one-frond inoculum growing at a 56-hour doubling time (DT) will produce 8 fronds (Table 1). A seven-day test with a one-frond inoculum growing at a 20-hour DT will produce 331 fronds (Table 1). The Test Protocol threshold growth rate therefore essentially advocates inhibiting growth in control cultures by approximately 95 %. This is not an optimal situation and will potentially confound the data, as the plants are likely physiologically stressed. Toxicity tests must be carried out under control conditions that are optimal or near optimal for the specific test organism, or as the CCME (CCME 1999) states, growth must be appropriate to the life cycle of the organism.

Recommendation: A light intensity of approximately 400 $\mu mol \cdot m^{-2} sec^{-1}$ would satisfy CCME requirements, and would produce an appropriate growth rate and final yield near maximal for the species.

Table 1. Final yield (frond number) in a seven-day test, with a one-frond inoculum.

Doubling Time (h)	Final Yield (frond #)	Reduction (%)
20	331	0
24	128	61
36	25	92
56	8	98

The Test Protocol advocates the use of 'final yield' as the parameter used to estimate toxicity. However, percent inhibitory concentration (ICp) values estimated from final yield data depend significantly on the duration of the toxicity test, and the growth rate of the control culture (Huebert and Shay 1993). Since both of these are variable (even within the Test Protocol), ICp values derived using final yield are of limited usefulness. ICp values could be adjusted simply by altering the duration of the test, or the growth rate of the control culture (for instance, by changing the light intensity), or both. For instance, copper toxicity could vary from 120 $\mu g \cdot g^{-1}$ dw to 2200 $\mu g \cdot g^{-1}$ dw, depending on control DT and test duration (Table 2). By contrast, estimates of toxicity based on growth rate are independent of the duration of the toxicity test, or the DT within the control culture (Huebert and Shay, 1993).

Recommendation: There is recognition that the use of growth rate as the response variable is 'scientifically preferred' (OECD 2006), and it is therefore recommended that this response variable be adopted within the Test Protocol.

Table 2. Internal copper content (μg·g^{-1} dw) required to produce an IC25 (based on final yield) at three growth rates and three test culture durations.

	Culture Time (d)		
Doubling time (d)	4	7	10
1	320	190	120
2	1900	1800	1700
3	2400	2300	2200

Source: Huebert et al. 1993

Test Protocol nutrient media contain EDTA:Fe in variable amounts and ratios. The EDTA:Fe is included in the various nutrient media because it is an absolute requirement for normal growth of duckweed. Unfortunately, the EDTA will also chelate metals. This is problematic, because metal toxicity depends on metal-ion activity, which is reduced by chelation. For instance, estimates of copper toxicity (as EC50) in *Lemna trisulca* have been found to vary from 3.6 to 22 μM Cu as EDTA varied from 9 to 27 μM (Table 3). Furthermore, the effect of EDTA will also vary depending on whether the EDTA is in excess of iron (Huebert and Shay 1992).

Recommendation: To minimize the effect of EDTA on estimates of metal toxicity, it is recommended that the Test Protocol specify that EDTA:Fe stock solutions be prepared with an excess of iron so that the EDTA is fully complexed, and that EDTA:Fe is used at as low a concentration as will support exponential growth through the duration of the test period.

Table 3. The effect of EDTA on estimates of copper toxicity.

EDTA (μM)	Cu EC50	
	External (μM)	Internal (μg·g^{-1}dw)
9	3.6	1310
27	22	1640
81	55	1570

Source: Huebert et al. 1993

A complementary approach for circumventing the confounding effects of EDTA could be to use the internal metal content rather than the external metal concentration for the estimation of toxicity. This would produce more consistent toxicity results (Table 3), although this approach would significantly increase costs. The utility of this approach is that it is independent of the chelating properties of the medium, and provides a causal connection between laboratory-generated data on toxicity, and field-collected data on metal content. It also provides a 'means of identifying the cause of toxicity' in metal mixtures. This latter advantage is especially critical given the relative lack of data on the effect of metal mixtures in aquatic organisms generally, and in aquatic macrophytes specifically.

The Test Protocol specifies several acceptable nutrient media for test cultures, and a nutrient medium for stock cultures, different from the others, that contains a complex mixture of organic supplements. However, duckweeds are not obligate heterotrophs, and therefore require no organic supplements for normal growth (e.g. Huebert et al. 1990).

Recommendation: Removal of the organic supplements would result in a considerable reduction in the difficulty of stock culture maintenance, and would be consistent with the recently published OECD protocol (OECD 2006).

The two duckweeds specified in the Test Protocol are both floating species. Their physiology differs from truly submerged species in important ways, such as in the presence of stomates, the presence of a cuticle, and the use of carbon dioxide from the atmosphere instead of from the water (Landolt and Kandeler 1987). There is also the complication of the air/water interface when interpreting toxicity data, as has been recognized in the recently published OECD protocol (OECD 2006). Duckweeds such as *Lemna trisulca*, on the other hand, are fully submerged and truly represent an aquatic organism.

Recommendation: It is recommended, therefore, that an alternative protocol be developed using the submerged *L. trisulca*, rather than the two floating species specified in the current Test Protocol.

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